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FEEDBACK IN DEVELOPMENT AND ITS EVOLUTIONARY SIGNIFICANCE

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INTRODUCTION

Two types of explanation are often applicable to biological phenomena;

1. Causal. E.g., "Air enters the lungs because of the movements of the ribs and diaphragm."

2. Teleological. E.g., "The function of the lungs is the oxygenation of the blood."

Teleological explanation implies a goal, some state whose attainment or maintenance is advantageous to the organism; the theory of natural selection makes this concept susceptible of mathematical and experimental study. Some workers would condemn all teleological explanation as unscientific, but in biology it merely implies that a phenomenon is selectively advantageous to the organism displaying it. Such knowledge is both scientific and useful, but it must be supplemented by a causal explanation of the mechanism by which the goal is maintained.

In many cases, the deviation from the goal is part of the causal system. For example, the size of the pupil is a controlling factor of the intensity of illumination of the retina, which has a definite goal. Disturbing factors may cause the controlled variable to deviate from its goal, but this deviation causes a change in the pupil which counteracts it and restores the goal. A system in which deviation from a predetermined goal causes controlling factors to oppose the deviation is called a negative feedback system. (As positive feedback in which a deviation causes changes tending to increase the deviation is of little importance, the word "feedback" alone usually connotes negative feedback.)

The idea of feedback is much older than its name; it was used by James Watt for the centrifugal governor. Recently its spectacular engineering applications to automatic control systems have popularized the engineering term. Biologists might prefer Cannon's earlier term "homeostasis," (1939), but this may suggest only the maintenance of a steady state, while feedback can guide the attainment of a predetermined state from a widely deviant initial one. Our use of the name is broader than that advocated by von Bertalanffy (1951), who would restrict it to mechanisms involving structural prearrangement, such as neuromotor mechanisms. His distinction

would be inconvenient for our purposes, and he suggests no alternative inclusive name.

For a discussion of the development of the concept and its general implications the reader is referred to Wisdom (1952), and to Wiener (1948), von Bertalanffy (1950, 1951), and Ashby (1952) for its detailed mathematical and theoretical treatment.

FEEDBACKS INVOLVING THE SIZES OF STRUCTURES

The mammalian kidney offers an extensively studied example of an organ whose size is involved in a feedback system. That one kidney hypertrophies after removal of the other is well known. Drury (1932) stunted one kidney of a rabbit by partial ligation of its artery; the other kidney grew to a larger size than controls. Mack and McGlothlin (1949) described a female cat in which one kidney had failed to form; the single kidney was roughly 1½ times the normal volume. Such facts suggest that total kidney weight may be a controlled variable, but other experiments refute the assumption.

Bollman and Mann (1935) showed that compensatory hypertrophy is increased by sewing the remaining ureter to the duodenum, so that excreted urea is reabsorbed. Hartman (1933) joined both ureters to an isolated segment of ileum; both kidneys increased greatly in size. Reader and Drummond (1925) observed renal hypertrophy in rats fed extremely high protein diets. MacKay *et al.* (1928) found a linear relation between renal weight and protein intake in young rats. In 1931, MacKay *et al.* added urea to the diet of rats and observed an increase in kidney weight. Allen and Mann (1933) fed high protein diets and diets containing urea to rats and rabbits both unilaterally nephrectomized and unoperated, and found an increase in kidney size in each case. In 1951, Addis *et al.* found a reasonably good straight-line correlation between kidney size and "calculated urea excretion work." Such results suggest that the size of the kidney is determined by the quantity of work demanded of it in maintaining the constancy of the chemical composition of the blood.

Additional evidence that kidney size is determined by its role as a controlling factor of blood composition is supplied by Fylling (1952), who found that the hydronephrotic kidney of a rat would recover only if the other ureter were ligated. Hinman and Butler (1923) similarly found that in dogs the repair of hydronephrosis required "the stimulus of compulsory function. ...If left in partnership with the normal kidney, which meanwhile has undergone compensatory hypertrophy, atrophy will replace the initial repair." Hinman (1923) attached one ureter of a dog to the duodenum; both kidneys hypertrophied markedly, but after about a year the operated kidney atrophied. Hinman's explanation, rephrased, is that the operated kidney is no longer an important controlling factor of blood urea level. Slight changes in its rate of activity therefore do not tend to be affected by feedback. Since most such changes will be toward less work, the organ tends to atrophy.

Gluecksohn-Schoenheimer (1943) discusses the mutation "Sd" in mice. In heterozygotes, one kidney is frequently absent while the other is normal; the single kidney does not begin to hypertrophy until after birth. That the kidneys of fetal rats may excrete urine has been shown by Wells (1946a) and Daly *et al.* (1947). They may be stimulated by urea injected into the mother, or by ligating her urogenital papilla (Wells, 1946b). However, Needham (1931) agrees with Bremer (1916) that in the rat, which has a highly permeable placenta, fetal nitrogenous excretion occurs almost entirely across the placenta. Apparently, then, renal hypertrophy in single-kidnied Sd mice is delayed until after birth because fetal kidney size is not a controlling factor in fetal blood composition, an assumption upheld by the fact that Sd homozygotes, with no kidneys, show no increased prenatal mortality.

A complication arises from the fact that a hypertrophying kidney continues to enlarge after blood composition has returned to normal. Karsner *et al.* (1915) and Allen *et al.* (1933) reported only transitory rises in blood urea and non-protein nitrogen after surgery decreasing the amount of functional kidney tissue. Welsh *et al.* (1944) found that after nephrectomy on a human patient the glomerular filtration rate and renal blood flow rose rapidly, but diodrast estimations of the mass of functional renal tissue rose slowly.

In 1922, Khanolkar demonstrated that not all the glomeruli of a rat function simultaneously. Richards and Schmidt (1924) observed the glomeruli of a living frog, and found blood flowing through only a fraction of them; the number was increased by the injection of adrenalin, caffeine or urea. Moore and Lukianoff (1929) found 44 per cent to 78 per cent of the glomeruli of a rabbit functioning at one time; this increased to 91–99 per cent after removal of one kidney, and remained at that level for several days. Such facts led Boycott (1932) to suggest that each functional unit of an organ only works periodically, with an optimum ratio between periods of work and rest; increased demand disturbs this ratio, which is restored by an increase in either the size or number of the units. In the kidney, the number of nephrons remains constant in hypertrophy (Arataki, 1926; Jackson and Shiels, 1927; Moore, 1929), but each nephron increases in size by both cellular hypertrophy and hyperplasia (Sulkin, 1949; Rollason, 1949). Presumably a larger nephron accomplishes more in the same working time.

The weight of the mammalian kidney is nearly proportional to the surface area of the body. In the rat, this is true at all ages between 35 and 760 days (MacKay and MacKay, 1927). In 1929, McIntosh *et al.* showed that to apply the same normal values for urea clearance, i.e., the hypothetical volume of blood from which all urea is removed in a given period to patients of various sizes requires the assumption that excretion varies directly as surface area. Experiments on rabbits (Taylor *et al.*, 1923), on rats (MacKay and Raulston, 1931), and on dogs (MacKay, 1932a) showed that the rate of urea clearance, bears a linear relation to kidney weight. MacKay (1932b) then pointed out that in man urea clearance and kidney weight are both proportional to body area. Since blood volume is roughly surface-proportional

(Best and Taylor, 1950) such a relation would maintain both blood urea concentration and the work rate of kidney tissue at constant values throughout growth. The kidney's role as a controlling factor in a feedback system would therefore serve to control its normal growth as well as its hypertrophy and atrophy. If these values were also constant in phylogeny, one would expect the ontogenetic and phylogenetic allometric constants of kidney weight to be the same, as they are (von Bertalanffy and Pyrozynski, 1952).

Kidney growth illustrates a class of mechanisms which we may call linked feedback systems. The primary goal of a linked system is a certain value of a physiological variable. A deviation of this variable from its goal is rapidly corrected by a change in the rate of activity of the controlling organ. This rate, per unit size, has an optimum which is the goal of a secondary feedback, and is of course controlled by the size of the organ. Many organs are involved in linked systems less thoroughly studied than that of the kidney; skeletal muscles are an obvious example.

Glinos and Gey (1952) found that cultures of rat liver cells grew more rapidly in serum from hepatectomized rats and in dilute normal serum than in undiluted normal serum. They also diluted the plasma of living rats by repeatedly drawing blood and reinjecting the washed cells in saline; histological examination of the liver showed numerous mitoses in a picture resembling liver regeneration. They postulate that the liver produces an unidentified substance which, in sufficient concentration, inhibits its growth. Unfortunately, it is not known whether its absence stimulates growth directly, or indirectly by causing increased production of itself. The latter alternative would make the system another linked system; the former would make it a morphogenetic feedback system; i.e., one whose goal is a value of a morphological variable, e.g., organ size, rather than a physiological variable.

Rose (1952a) postulates that several types of differentiation are possible for each embryonic cell, but products of chemical reactions involved in each will prevent later cells from pursuing the same course, forcing them into alternative paths. Living adult brain, blood and heart cells in the media in which young frog embryos were reared prevented the differentiation of similar cells (Rose, 1952b).

Weiss (1947) has advanced "a template concept of growth, with molecules of complementary configuration acting reciprocally as moulds for each other's synthesis.... Growth rates, according to this concept, would be governed by the concentrations in which the two complementary systems would be present and by the extent to which they would become conjugated and thereby inactivated as specific catalysts. Evidently, if some of... these compounds are liberated from their source cells, their concentration in the medium could exert a growth-controlling influence on distant homologous cells. It is not inconceivable that many cases of 'compensatory hypertrophy' which cannot be explained by the 'functional overloading' of the residual tissues, go to the credit of such systematic balances."

The evidence for this hypothesis cannot be reviewed here; the reader is referred to Weiss (1947, 1952), Weiss and Wang (1941) and Ebert (1951). However, this work and that of Rose make it plausible that differentiation and perhaps growth are controlled by their own by-products, which retard them as they approach their goals; the same feedback would restore the goals after a disturbance.

Where one can evolve, however, a linked feedback must have a selective advantage over a morphogenetic feedback, since the latter cannot dependably adjust organ size to a variable environment. Linked feedbacks cannot operate until their controlling organs are functional, while morphogenetic systems need involve no structural complexity above the chemical level. One would therefore expect to find morphogenetic feedback in early embryos, and linked systems controlling later growth. There is little concrete evidence that morphogenetic feedbacks operate in post-embryonic development.

A third category, simple (i.e., unlinked) feedbacks with physiological goals, also exists. In such systems, the size of a structure directly controls some physiological variable, with no secondary goal as in a linked system. The ecological rules of Bergmann (individuals of a warm-blooded species are smallest near the equator) and Allen (the appendages of a warm-blooded animal are smallest in the coldest parts of its range) suggest such systems with size and proportions as controlling factors, and a certain rate of heat loss as the goal. Hesse, Allee and Schmidt (1951) describe several experiments on mice, rabbits and fowl showing an increase in body size and a decrease in ear, foot and tail length at low temperatures. Such feedbacks therefore do exist, although in some forms size and proportions are relatively fixed genetically (Dobzhansky, 1941; Huxley, 1943) presumably leaving the control of heat loss to other mechanisms.

FEEDBACKS INVOLVING THE FORMS OF STRUCTURES

More than one writer has claimed that all "goal-seeking" behavior in organisms can be explained by the principles of feedback. These principles show that if the variables of a system bear certain mathematical relations one to another, one of them will tend toward a certain value and return to it after a deviation. To say that a system is explained by feedback without demonstrating the required mathematical relations is merely to extend the definition of feedback, saying in effect, "All goal-seeking systems can be explained by the principles of feedback because we intend to call any principle explaining such a system a feedback principle." This remarkable nomenclature has the questionable merit of wide acceptance; as the reader will have observed, the writer has adopted it in this paper.

To explain a system by the *already known* principles of feedback requires every variable involved to be a single number, such as an area, rate, or ratio, which can be inserted in one of the model equations from which the principles are deduced. The examples in the previous section have been selected to meet these criteria.

Systems satisfying our broad definition of feedback may involve the forms of structures; but form can usually be described only in terms of several numerical variables or non-numerical relations. Limb regeneration and regulative embryogeny are examples of such feedbacks. Carey (1921) inserted a side tube into a dog's bladder and injected increasing volumes of liquid over a period of weeks. The bladder musculature changed histologically into striated muscle resembling heart muscle, which contracted rhythmically under tension. In his final experiment, the bladder was able to excrete 50,000 ml. in ten hours. Such changes clearly were controlling factors of the pressure in the urinary system, but there is no known way to describe them quantitatively. Therefore, although the name "feedback" is properly applicable to the phenomenon, it cannot be explained by any yet known principles of feedback.

In some cases, however, a complicated shape is determined by a single numerical variable. The work of d'Arcy Thompson (1942) is a fruitful source of examples. The writer has found that encrusting portions of the sponge *Microciona prolifera* are usually roughly two mm. thick, while the branches which extend upward vary in diameter from about four to almost ten mm. The larger branches, however, have dead cores surrounded by a living layer about two mm. thick. The living portion of the sponge thus has a roughly constant surface-volume ratio, and a feedback involving this numerical variable may exert a determining influence on the form of the animal. Oysters grown in mud are often long and narrow, presumably because the mantle will not be extended into mud, and so can deposit new shell only at the exposed end. The controlled variable "muddiness of mantle" and its controlling factor "degree of extension of mantle" jointly determine the shape of the shell.

Certain tissues develop a pattern corresponding to the pattern of stresses to which they are subjected. Rollhauser (1953) found that the birefringence of the gastrocnemius tendon of the guinea pig decreased after freezing of the sciatic nerve, was regained as the muscle recovered its function, and was increased by unusual exercise. Weiss (1938) showed that embryonic cartilage differentiating *in vitro* under mechanical stress developed a trajectorial pattern, cells lining up along lines of maximum strain.

Bone, however, is the most striking example. The trabeculae of the human femur lie on lines of strain, but they do not acquire this pattern until the child begins to walk; in *pes equinus*, a deformity in which the heel does not touch the ground, the calcaneum never forms the usual sets of trabeculae (Murray, 1936). On the other hand, "prolonged recumbency or immobilization cause decalcification and atrophy of bone... The transverse trabeculae which are the last to be laid down in a growing child are the first to be removed." (Watson-Jones, 1940). Glegg (1953) studied the deposition and removal of radioactive phosphorus in bones and teeth, and suggests that its movements may be due to the effects of tension and pressure on crystal solubility. Such a hypothesis may eventually permit

the explanation of trajectorial patterns in terms of a feedback whose goal is a minimum amount of shearing force.

These illustrations show that the belief that all organic feedbacks can be explained by present-day feedback theory is unproved, but is an expression of partly justified optimism.

THE EVOLUTIONARY SIGNIFICANCE OF DEVELOPMENTAL FEEDBACK

Genetic changes may affect either the goals of feedback systems or the efficiency of their controlling factors. These two different effects should lead to two different types of selection.

If a genetically determined physiological state is the goal of a feedback, it will be attained in nearly all environments, and there will be no variation in the selective advantage of the determining gene. Gruneberg (1939) supplies an example. The blood haemoglobin concentration of mice of the genotype W^2W^2 is roughly one-third that of normals. Under low air pressure, both normals and W^2 homozygotes increase their haemoglobin and red cell concentrations, but the difference between them persists; on return to normal pressure, the bloods of both genotypes regain their respective "normals" equally rapidly. Both recover from haemorrhage in the same time. Blood oxygen tension seems to be the controlled variable in both genotypes, but its goal is much lower in the mutant, which is therefore at a selective disadvantage unaltered by environment.

On the other hand, a mutation affecting a controlling factor will bear different degrees of advantage in different environments. In Gruneberg's (1947) cartilage anomaly in rats, the abnormally thickened ribs become fixed in an inspiratory position, preventing the lungs from exerting their controlling influence on blood oxygen. The result is an extreme oxygen deficiency, usually lethal, but the disadvantage could presumably be reduced or increased by changing the atmospheric oxygen tension.

The disturbing factors of a feedback system may lie within the organism. Landauer (1937) discusses the secondary effects of loss of feathers in frizzle fowl. The B.M.R. rises, the thyroid enlarges without producing symptoms of hyperthyroidism, and the heart, kidneys and digestive organs enlarge. These effects may be prevented by keeping frizzle fowl in heated buildings, and imitated by partly denuding normal fowl. Here, mutation has disturbed the physiological goals of various feedbacks, and morphological changes have acted with some success to correct the deviations. Such changes may be called corrective pleiotropy.

Corrective pleiotropy is of considerable evolutionary importance; it ensures the physiological balance of organs in spite of mutations affecting such things as size and the insulating efficiency of the integument. Without feedback systems correcting such disturbances, evolution would have to wait for highly unlikely combinations of simultaneous mutations. Many cases of simple evolutionary allometry are undoubtedly due to corrective pleiotropy.

An important problem in modern biology is to explain in a non-Lamarckian manner what Waddington (1953a) calls "pseudoexogenous adaptation," i.e., adaptation which presumably could be directly caused by the environment but which in fact occurs without an environmental stimulus; e.g., the thickened soles of the feet of the human infant. Two major hypotheses have been advanced. The first postulates the "stabilizing selection" of mutations which imitate a direct environmental effect. It has been discussed at length by Kirpichnikov (1947) and Schmalhausen (1949), but was introduced earlier as the "Baldwin effect" (Simpson, 1953). It has been criticized by him and by Waddington (1953b) who point out that either the environmental stimulus must be held to cause the required mutations, which is neo-Lamarckism and already sufficiently refuted, or the process depends entirely on coincidence and adds nothing to the neo-Darwinian theory.

The more recent hypothesis is Waddington's "genetic assimilation," (1942) here presented in his own words:

> If it is an advantage, as it usually seems to be for developmental mechanisms, that the response should attain an optimum value more or less independently of the intensity of stimulus received by a particular animal, then the reactivity will become canalized, under the influence of natural selection.... Many different agents, including a number of mutations available in the germplasm of the species, will be able to switch development into it; and the same considerations which render the canalization advantageous will favour the supersession of the environmental stimulus by a genetic one.

As experimental evidence, Waddington (1952, 1953a) describes a population of Drosophila in which, after several generations of suitable selection, crossveinless forms became common at normal temperatures although initially occurring only after exposure to 40°C.

Most exogenous adaptations adapt the organism specifically to the stimulus producing them; the environment supplies a disturbing factor and the morphological change is a controlling factor in a feedback with a physiological goal. Waddington's experiment is one of the rare cases of an advantageous exogenous modification not of this type; and the selective advantage of crossveinlessness is a purely artificial condition of his experiment. But both stabilizing selection and genetic assimilation demand the assumption that "it is an advantage...that the response should attain an optimum value more or less independently of the intensity of the stimulus received." That is, the "best" value of the controlling factor is independent of the disturbing factor! Such genetic fixation is comparable to being sewn into one's winter underwear. It may well be advantageous for a switching gene to turn development into a certain path, but environment must affect its progress and determine its destination. The sole of the fetal foot is thickened because of genetic fixation, but further thickening, far from leading to an optimum independently of the environment, is sensitively responsive to it.

The function of almost any organic structure is the maintenance of some physiological conditions in the organism. This is obvious for most vis-

cera, and the rule can be applied, perhaps cumbersomely, to sensory and locomotory organs and even color patterns. But physiological conditions in the organism are influenced by the environment; they are determined, not by organs alone, but by organs and the environment acting jointly. If the environment vary, physiological conditions cannot be constant unless organs too can vary.

Throughout evolution, organisms have come to live in progressively more variable environments. This progress has required the ability to maintain the constancy of many physiological conditions, which demands the increased variability of many other factors; not, however, the random, functionless variation of primitive organisms, but a controlled variability precisely co-ordinated with that of the environment. The rapid, transitory changes in organ activity so well known to physiologists illustrate such co-ordinated variation. As natural selection is concerned only with the efficiency with which functions are performed, a morphological property of a structure is important only if it affects its function; hence, morphological variability too has been advantageously applied to protect the organism from the vicissitudes of its environment.

SUMMARY

A feedback system is one in which the deviation of a certain variable from a certain value causes controlling factors to oppose the deviation. The name denotes, not a class of explanatory principles, but a class of phenomena to be explained. Explanatory principles, mathematical in form, have been developed for certain general types of such systems. These are inapplicable to feedbacks involving the forms of structures, but can be applied to those which involve only quantitative variables.

Three categories of quantitative feedback influence morphology: (1) Morphogenetic feedback, in which the size of a structure is the controlled variable; (2) Simple feedback in which the goal is a physiological state, controlled by variations in morphology; (3) Linked systems, in which a physiological state is maintained by changes in the rate of activity of a structure, while the rate per unit size is kept constant by changes in size. (2) and (3) ensure physiological stability in a variable environment, and tend to supersede morphogenetic systems in late development.

The selective effect of a mutation changing the goal of a feedback will be the same in any environment in which the goal can be maintained, but that of a gene affecting a controlling factor will vary with the environment.

Developmental feedback causes corrective pleiotropy, maintaining the physiological balance of organs in spite of mutations tending to disturb it.

Genetic fixation may ensure that a developmental process is begun, but it cannot determine the end-point, which must adjust itself to the environmental conditions to which it is an adaptation.

The environmental independence of higher organisms implies the independence only of certain physiological variables, and this is the result of

the readiness with which precise and co-ordinated changes are imposed on many other variables, physiological and morphological, by the environment.

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A MECHANISM OF GENE INTERACTION

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No experiment has yet been designed which catches the gene in the act of doing something. Further, at the level of the "immediate gene product," it is extremely difficult to decide on the boundaries of the gene, or to decide if genes do exist. Although it is not now possible to construct a satisfactory model of the gene and its immediate product, it is possible to give a coherent picture of the class of non-allelic gene interactions, that is, the class in which the properties of a double mutant are different from the sum of the properties of the single mutants into which it may be resolved. This paper is an attempt to give such a picture.

In this discussion the "gene" refers to a factor whose presence is correlated with the appearance of an experimentally distinguishable pattern of characteristics and which is transmitted in Mendelian fashion. In this discussion, two "pseudoallelic" genes are considered alleles when the mutation of either affects the same set of phenotypic characteristics. This definition is adopted because it makes possible a physiological interpretation of gene action; there are probably no innate advantages of the definition for statistical or evolutionary purposes. Gene interaction as here used refers only to the interaction of the genes as defined above, not to possible intra-genic effects such as the position effect phenomenon seen in cases of pseudoallelism.

SYSTEMS IN BIOCHEMISTRY

The single-step reaction is the basic process of biochemistry. Perhaps the first principle of biochemistry is that biological reactions occur in single steps, each step resulting in rather minor modification of the reacting molecules, the reactions catalyzed by separate, specific enzymes. The sum total of these small changes makes up the metabolism of an organism.

Systems of enzymes working in concert are called "multi-enzyme systems" (Dixon 1949). Enzymes in such systems are linked together by a common product-reactant as

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C;$$

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E_1 and E_2 are linked together by the common substance B. In a like manner, enzymes E_3 and E_4 are linked by the common hydrogen carrier F.

$$DH + F \xrightarrow{E_3} FH + D$$

$$FH + G \xrightarrow{E_4} GH + F$$

A "bag of enzymes" containing such multi-enzyme systems could successfully carry out many metabolic functions, the specificity of the individual enzymes organizing the system. Systems may be organized *in vivo* also by orientation on structures such as mitochondria; the juxtaposition of protein structures makes possible their coupling by electron transport, the whole system serving as a conductor (Leach 1954).

All metabolites are worked on by at least one multi-enzyme system during their passage through the organism. The relative affinities for the same metabolite (substrate) of enzymes leading into different systems gives direction to metabolism; the relative activities of E_5 and E_6 determine the metabolic fate of A.

$$\begin{array}{l} E_5(k_5)\ B \xrightarrow{E_7} G \xrightarrow{E_8} H \\ \quad\nearrow \\ A \\ \quad\searrow \\ E_6(k_6)\ C \xrightarrow{E_9} D \xrightarrow{E_{10}} E \end{array}$$

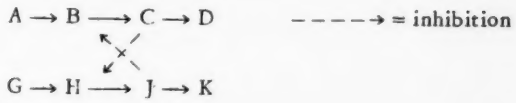
Competition between enzymes for the same substrate therefore provides a mechanism for the interaction of multi-enzyme systems; the flux of the system B → G → H is *not* independent of the flux of C → D → E.

Related multi-enzyme systems may be linked by common enzymes as demonstrated by Myers and Adelberg (1954) for the synthesis of isoleucine and valine:

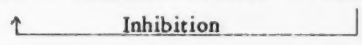
$$\begin{array}{lll} A \rightarrow B \rightarrow C \rightarrow D & & \text{(path 1)} \\ \qquad\quad | \quad | & & \\ \qquad\quad E_1 \quad E_2 & & \\ \qquad\quad | \quad | & & \\ G \rightarrow H \rightarrow J \rightarrow K & & \text{(path 2)} \end{array}$$

Enzyme E_1 catalyzes both reaction B → C in path (1) and H → J in path (2); E_2 catalyzes C → D in path (1) and J → K in path (2). It is evident that the rate of D production depends on the relative affinities of E_1 for B and H, on E_2 for C and J, on the rate of B production and on the rate of H production, on the amounts of E_1 and E_2 and on the degree of saturation of the enzymes *in vivo*. If the enzymes are saturated there will be a definite competition between B and H and between C and J for their respective enzymes. Both systems are obviously interrelated by this interaction; the rate of production of D is not independent of the rate of production of K.

An additional mechanism for the interaction of multi-enzyme systems by mutual inhibition has been described by Delbruck (1949);



In this case the mutual inhibition of parallel reaction systems provides the mechanism for interaction. Although linkage of enzymes by a common reactant is a most evident model for the *production* of substances and for the coupling of reactions for energy transfer, mechanisms of inhibition are satisfactory for the control of the flux of reaction chains. Such mechanisms may operate within chains by feedback mechanisms as well as across chains. Thus, in the chain



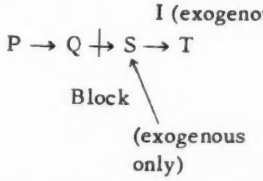
oxalacetic acid is a potent inhibitor of the succinate-fumarate reaction *in vitro*. If the inhibition occurs *in vivo* this would amount to control of the rate, or flux, of a reaction chain by a feedback mechanism; an accumulation of oxalacetic acid would inhibit its own formation. Since biochemists have not been notably successful in their search for such regulatory mechanisms, it is still possible that other types of interaction mechanisms will be discovered.

The interaction of multi-enzyme systems may act as a mechanism for the control of the velocity of multi-enzyme systems *in vivo* even though the mass-action principle does tend to keep reacting series in step. There is evidence that biological systems do use inhibition mechanisms as control devices and under the proper circumstances (correct enzyme-inhibitor dissociation constant K_i—see appendix) a change in the concentration of an inhibitor might be as effective as a comparable change in substrate concentration in altering the velocity of an enzymatic reaction.

Cases in which normal metabolites serve as inhibitors do exist and these have been made the basis of a theory of biochemical regulation (Woolley 1952). Sodium and potassium ions are mutually antagonistic in the systems of mammalian physiology, for bacterial growth and for certain isolated enzyme systems. Antagonisms between pairs of similar amino acids have been reported; several groups of lactic acid bacteria have their serine requirement conditioned by the amount of threonine present in the medium. A lysineless strain of Neurospora is inhibited specifically by arginine, mutants requiring cytidine are competitively inhibited by adenine. Mutants requiring threonine are inhibited by methionine; sulfanilamide is required by certain mutants of Neurospora to overcome the toxicity of the para-aminobenzoic acid produced endogenously (Zalokar 1950).

The inhibitions displayed by mutant strains must be magnifications of inhibitions normally present in the wild-type. Consider the case of a mutant-

requiring substance (S), the mutant competitively inhibited by the related substance (I) supplied endogenously or exogenously.



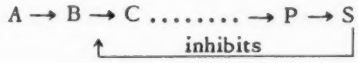
(I) must inhibit (S) utilization since the inhibition is competitive (i.e. related to (S)) and since, experimentally, (S) is supplied exogenously to the mutant rather than synthesized. Assuming that the effect of the mutation is on the systems involved in (S) formation and assuming as the simplest case that the mutation has not simultaneously affected the systems concerned with (S) formation and utilization, the systems for (S) utilization must have been susceptible to inhibition by (I) before the mutation since they have not been changed by the mutation. The inhibition observed in the mutant would then be a magnification of a situation normally present in the wild-type. The following hypothetical scheme could account for the failure of the wild-type to be inhibited by concentrations of (I) which do inhibit the mutant.

Wild-type I (exogenous or endogenous)

$$P \rightarrow Q \rightarrow S \rightarrow T$$

In the wild-type the concentration of (S) can be increased endogenously to overcome the effects of (I), in the mutant the only way of overcoming inhibition by exogenous (I) is by the addition of (S) exogenously.

Several cases of interactions within a single multi-enzyme system have been described (Adelberg and Umbarger 1953, Cohn and Monod 1953, Strauss (1955). In these cases the product of a reaction series prevents the accumulation or formation of precursors in the series or of the product itself. Thus acetate inhibits the accumulation of pyruvate by acetate-requiring mutants of Neurospora, valine inhibits the accumulation of a valine precursor by valine-requiring bacterial mutants; methionine inhibits the formation of the system forming methionine in bacteria (non-mutant). In the case of the acetate-requiring mutants acetate seems to be a true inhibitor of pyruvic acid accumulation. The data can be represented by the following scheme:



This inhibition of precursor accumulation helps explain the essentially normal physiology of many biochemical mutants fed a sufficient quantity of growth substance. The addition of growth factor reduces the accumulation of precursors, thereby preventing the interaction of these precursors with other systems.

The best known case of regulation within a multi-enzyme system is the Pasteur effect. As Racker (1954) points out, the effect is most likely due

to an inhibition of the phosphorylation of glucose (hexokinase reaction) by some product of glucose oxidation.

More general cases of regulation by inhibition are known to the biologist. The rate determining mechanism of the heart is regulated both by an accelerating mechanism and an inhibiting mechanism (the vagus). The discharge of the vagus acts as a continuing brake on the heart. More complex cases of inhibition occur in the central nervous system. The endocrine system of vertebrates acts to keep the organism in a state of "balance" as a result of the interaction of antagonistic substances. The pancreatectomized, hypophysectomized animal mimics the normal. Antagonism between testosterone and estrone or between progesterone and estrogen can be demonstrated (Woolley 1952) and the endocrinologist arrives at a "balance" theory of sex determination formally similar to that of the geneticist (cf. below).

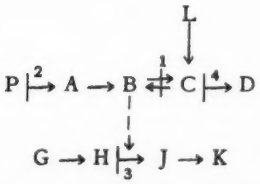
The regulation of growth processes in plant material may be a result of the interactions of growth and anti-growth substances (deRopp 1954). Plants subjected to continuous gamma irradiation display unusual growth responses; roots are produced by stem tissue and numerous tumors are produced (Gunckel and Sparrow 1954). Such results suggest the destruction of a normal growth regulator (inhibitor) by the irradiation treatment.

In summary: The biochemical reactions that make up metabolism are arranged in multi-enzyme systems. These systems may be coupled for synthesis by means of common reactants. The series of multi-enzyme systems that operate in an organism are not independent nor are their rates controlled solely by the operation of the mass-action law. Multi-enzyme systems interact by various mechanisms and this interaction serves to control the whole of metabolism. The metabolism of a normal organism is in a balanced state determined by the final effect of the individual interactions on its multi-enzyme systems.

SYSTEMS IN GENETICS

Genes act by controlling the rates of the biochemical step reactions. This relationship between the gene and enzymatic reactions is not related to the validity of the "one gene – one reaction" hypothesis or to the question of the nature of the immediate gene product. As Pontecorvo (1952) implies, critical tests of the "one gene – one enzyme" hypothesis are at present impossible because of the vague outlines of the single gene. Notwithstanding this difficulty of definition, it is possible to demonstrate experimentally that a gene change is directly correlated with an heritable change in the velocity of a particular biochemical step reaction (Goldschmidt 1938 , Wright 1941, Beadle 1945, Horowitz and Owen 1954).

Step reactions are organized into multi-enzyme systems, and if these reaction systems interact it is apparent that the genes controlling the component reactions of the interacting systems must themselves appear to interact. Assume the following situation:



Compounds D and K are required for growth and the two reaction chains interact by inhibition as indicated by the dotted arrow. Then mutation (1) would create a requirement for exogenous K, mutation (2) would act as a genetic supression of (1), mutation (3) would mimic the effects of (1) and (4) would result in a pleiotrophic effect creating a requirement for D and K. Obviously, similar models can be designed to demonstrate suppression or modification of genic effects. Several such schemes are given by Beadle (1945) as illustrations of epistasis and of interactions giving rise to altered F_2 ratios. *The common feature of such schemes is that the gene interaction obtained is not in any sense an interaction of the genes themselves, but is rather an interaction of the gene-controlled step reactions in non-genic parts of the organism.* With the possible exception of position effect phenomena the cases of gene interaction so far described are probably of this type; the interaction may be at the level of enzyme formation reactions as well as at the level of the reactions of intermediary metabolism. There is no compelling reason to suppose, for example, that suppressors are (new) genes which have taken over the function of mutant genes.

Genetic sex determination is an important case of gene (or chromosomal) interaction. According to the hypothesis of Bridges (1925) the ratio of the autosomes to the X chromosomes of Drosophila determines the maleness or femaleness of the resulting fly. This is the balance concept of sex determination and is not limited to a single organism in its applicability. This "genic balance" concept of sex determination is similar to the hormonal balance concept of the endocrinologist as pointed out above. Both concepts are aspects of the same phenomenon at different levels. The antagonistic nature of the balance involved can be seen in such diverse material as in the experiments of Witschi (1939) on the expression of sex in salamanders with grafted partners and in the experiments of Moewus (1950) on sexual "valence" in Chlamydomonas.

A series of mutants (*td*) unable to transform indole to tryptophan is known in Neurospora (Yanofsky 1952). These mutants are deficient in their ability to form the enzyme tryptophan desmolase. In the presence of a suppressor gene (*su*) one of the *td* strains (td_2) is able to form tryptophan desmolase although the amount of enzyme formed by the suppressed mutant is less than that produced by the wild-type. However, the *su* gene does not induce the formation of tryptophan desmolase when combined with the *td* allele td_1. *This indicates that the suppressor gene alone is not responsible for the formation of tryptophan desmolase but rather that there is an interaction between the* td_2 *allele and* su *resulting in enzyme formation.* The

suppressor therefore "does not appear to be a duplication at another locus of the normal allele" and the suppression must therefore be due to an interaction of the type discussed above since at least two non-allelic genes (td_2 and *su*) are required for the effect. Furthermore the suppressed mutant $td_2 su$ grows less in the presence of a given amount of tryptophan than does the non-suppressed $td_2 su^+$, indicating that the action of the *su* gene has an effect other than the enhancement of tryptophan desmolase formation, again as required by the formulation of gene interaction discussed above; a similar phenomenon has been observed with suppressed and non-suppressed acetate-requiring mutants (Lein and Lein 1952).

If genetically-induced nutritional requirements are (in some cases at least) a result of the "imbalance" of reaction systems leading to internal inhibition, and if suppressor action involves a precarious restitution of the normal situation by introduction of a second lesion, then it should be possible to suppress the growth-substance requirement of a mutant by genetic combination of the mutant with another, if the two mutants are properly related biochemically. This rationale was used by Wagner and Haddox (1952) to explain the loss of the requirement for pantothenic acid in a pantothenicless-tyrosineless double mutant. A related case of suppressor action was analyzed by Strauss and Pierog (1954). An acetate requiring mutant (*ac*) is inhibited by glucose. A second mutant (*sp*) has no growth requirement but is partially deficient in pyruvic carboxylase activity. The double mutant, *ac sp*, is not inhibited by glucose and grows slightly in the absence of acetate. It seems that as a result of a block in the oxidative decarboxylation of pyruvic acid the *ac* mutants form an inhibitor chemically related to acetaldehyde. The *sp* gene reduces the rate of acetaldehyde formation from glucose by lowering pyruvic carboxylase activity. This removes the inhibition but permits some growth since acetaldehyde can be oxidized to acetate. In this case then, the suppressor gene (*sp*) acts in a manner consistent with the mechanism proposed for gene interaction, that is, by controlling the interaction of two enzyme systems. The imbalance leading to inhibitor accumulation caused by the first mutation (*ac*) is compensated for by the second mutation (*sp*) preventing the accumulation of inhibitor; the second mutation therefore restores the organism to a condition which more or less mimics the normal.

The requirement of a biochemical mutant for a particular growth substance is not a basic property of the mutant gene but can be altered by changing the genetic environment. (Haskins and Mitchell, 1952, Mitchell and Mitchell 1952, Newmeyer and Tatum 1953.) In the investigation of Mitchell and Mitchell a suppressor gene in one environment acted as an inhibitor in another and in yet another genetic environment appeared to change the point of the genetic block. This behavior is not precisely understood on the biochemical level, but it seems likely that the explanation when it comes will be on the basis of the interaction of multi-enzyme systems. The suppressed strains of Neurospora so far investigated do not usually reach the wild-type level of the character suppressed but merely approach it in various degrees.

This failure of complete suppression is understandable on the basis of the above ideas; the suppressed strains carry two biochemical lesions rather than the one of the original mutant.

In summary: The various types of non-allelic gene interactions; modifier action, suppressors, epistatic genes, sex determination, are a result of the intra- and inter-actions of multi-enzyme systems. These interactions can involve inhibition as a control mechanism and are only one class of a number of biological phenomena regulated by inhibition. The wild-type may be defined as an organism whose gene-controlled multi-enzyme systems are in a state of balance. A system is balanced between alternate states when it is able to develop on a minimum number of substrates with minimum accumulation of intermediate metabolic products and with the maximum stability to change consistent with life, that is, when the system resists an increase in the number of accumulations or requirements in response to an external factor as compared to possible alternate systems.

It should be possible to define more precisely the nature of the inhibitions responsible for gene interaction, growth regulation etc. as more cases are studied. Other mechanisms of interaction will probably be discovered; it is not likely that the mechanisms of mutual inhibition now recognized exhaust the possibilities. Finally it should be possible, eventually, to predict the biochemical properties of a mutant containing (n) mutant genes from the properties of the single mutants involved.

SUMMARY

The properties of multi-enzyme systems are discussed and a mechanism of coupling such systems by means of mutual inhibition is suggested. Coupling by inhibition is a characteristic of varied types of biological phenomena. Genes control the component step reactions of multi-enzyme systems. Gene interaction is the result of the interaction of the multi-enzyme systems controlled by the genes rather than by interaction of the genes themselves.

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APPENDIX

The comparative efficiency of slight changes of substrate or inhibitor concentration for the control of the velocity of an enzyme catalyzed reaction. Assume the enzymatic reaction: $E + S \rightleftarrows ES \rightarrow E + P$; $E + I \rightleftarrows EI$ and assume that the Lineweaver-Burk formulation for competitive inhibition adequately describes the reaction. That is

$$\frac{1}{v} = \left[\frac{1}{V_m}\left(K_m + \frac{K_m(I)}{K_i}\right)\right]\frac{1}{S} + \frac{1}{V_m}$$

where v = velocity of the reaction; S = substrate concentration; I = inhibitor concentration; V_m = maximum velocity; K_m = Michaelis-Menten constant i.e. $[E][S]/[ES] = K_m$; K_i = enzyme-inhibitor dissociation constant $[E][I]/[EI]$

then at constant [I]

$$\frac{-\partial v}{v^2} = \frac{-\partial S}{S^2}\left[\frac{1}{V_m}\left(K_m + \frac{K_m(I)}{K_i}\right)\right]$$

$$\frac{\partial v}{v} = \frac{\partial S}{S}\,\frac{v}{S}\left[\frac{1}{V_m}\left(K_m + \frac{K_m(I)}{K_i}\right)\right]$$

and at constant [S]

$$\frac{-\partial v}{v^2} = \frac{K_m}{K_i S V_m}\,\partial I; \quad \frac{-\partial v}{v} = \frac{\partial I}{I}\,\frac{v}{S}\,\frac{K_m I}{K_i V_m}.$$

Considering only absolute values, in order to produce the same percentage change ($\partial v/v$) in velocity by varying inhibitor concentration as by varying substrate concentration

$$\frac{\partial S}{S}\,\frac{v}{S}\left[\frac{1}{V_m}\left(K_m + \frac{K_m I}{K_i}\right)\right] = \frac{\partial I}{I}\,\frac{v}{S}\,\frac{K_m I}{K_i V_m}$$

$$\frac{\partial I}{I} = \frac{\partial S}{S}\,\frac{\dfrac{v}{S}\dfrac{K_m}{V_m}\left(1 + \dfrac{I}{K_i}\right)}{\dfrac{v}{S}\dfrac{K_m}{V_m}\quad\dfrac{I}{K_i}}$$

or

$$\frac{\partial I}{I} = \frac{\partial S}{S}\left(1 + \frac{K_i}{I}\right);$$

that is, to give the same percentage change in reaction velocity the inhibitor concentration must change by a percentage equal to $(1 + K_i/I)$ times the percentage change in substrate concentration. When K_i is very low (with a potent inhibitor) $(1 + K_i/I) \cong o$ and a change in inhibitor concentration is about as effective as an equivalent percentage change in substrate concentration for regulating reaction velocity.

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MUTATION AND QUANTITATIVE VARIATION

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Almost all our knowledge of the origin of new variation by mutation comes from work on individual genes with lethal or visible effects. The extent to which new variation arises in continuously variable characters has received little attention. Gustafsson (1953) found that irradiation of barley produced variants of practical value though most of these may be simple Mendelian segregants. More recently, Scossiroli (1953) and Buzzati-Traverso (1953) have shown that new variation induced by radiation can be utilized by either natural or artificial selection to give striking changes in a population. The former was using two lines selected by Mather for the number of sterno-pleural chaetae until there was no further response. By irradiation and selection, he was able to change the mean of the up line from its previous plateau of 26 bristles to 44 bristles in 17 generations of radiation and selection. He was however unable to change the down line. It seemed possible to us that in the up line there had remained some unfixable genetic variation which had been released by the radiation perhaps by the production of chiasmata in unusual regions. Stimulated by this work, we decided to investigate on a small scale the effects of radiation in a strain in which genetic variation had been reduced to a low level by inbreeding.

In our other work on abdominal chaetae (Clayton, Morris and Robertson, in press) we have used as base population a stock which shortly after captivity in the wild has been kept in a population cage with average numbers around 5000. Mr. B. K. Sen, working on egg production in Drosophila, made several inbred lines from this by continued full-sib mating and one of these was chosen for the radiation work because, of the surviving lines, it had the least variance in the count of abdominal chaetae. It had then been inbred for 28 generations. Four selection lines were started in two groups. The two irradiated lines were given 1800 r of X-rays as adults each generation and the two control lines were not treated. Within each group, one line was selected upwards for the total number of chaetae on the fourth and fifth abdominal segments and the other downwards. The selection procedure was the same in all cases—25 ♂ and 25 ♀ were measured and the extreme 10 of each sex chosen as parents of the next generation. Culture was in half-pint bottles. The selection was continued for 17 generations.

The current effect of the radiation on lethals was not checked but at the end of the experiments, a sample of third chromosomes was made homozygous, by the usual technique, for each of the four lines. The numbers of chromosomes lethal and non-lethal when homozygous are given in table 1.

There is no doubt about the accumulation of lethals in the irradiated stocks. In a population of this small size, the equilibrium between mutation

TABLE 1

		Lethal	Non-lethal
Control	High (HC)	2	32
	Low (LC)	0	32
Irradiated	High (HR)	58	9
	Low (LR)	29	9

and selection is reached fairly quickly. If the heterozygotes are not at a disadvantage, Wright (1937) has shown that lethal recessives reach an equilibrium frequency of $\mu\sqrt{2\pi N}$ where μ is the mutation rate per generation and N the effective population size. If we take the actual size to be the effective size in our case (although the latter may in fact be less), the equilibrium frequency is about 11μ and should therefore have been reached during the experiment. The observed frequencies of lethal chromosomes are 3 per cent and 83 per cent in the control and irradiated lines respectively. Taking these as the equilibrium figures and allowing for chromosomes carrying more than one lethal, the mutation rate per generation works out at 0.3 per cent and 16 per cent, which is not unreasonable.

The effects of the selection are given in table 2 in which, to smooth out differences between generations due to the small numbers measured, the

TABLE 2

Generation	HC	LC	HR	LR
1–5	28.93	28.88	29.65	29.26
6–10	29.52	29.29	31.20	29.18
11–17	28.99	28.93	32.26	28.69
17A	29.02	28.72	31.64	28.30

generations are given in three groups 1–5, 6–10 and 11–17. The mean count of the two sexes has been taken. It had seemed possible in the early stages of the work that the radiation might be having a direct effect on the mean. This could arise because of the smaller number of eggs hatching in the radiation lines due to dominant lethals. For the last generation, 17A, there was therefore neither selection nor radiation and 50 flies of each sex were counted instead of 25.

The control lines change remarkably little during the course of the experiment. HC is higher than LC in the last two entries, the differences being 0.06 ± 0.36 and 0.30 ± 0.54 respectively. The radiated lines do show a slight drop of 0.50 bristles in 17A from the previous entry in agreement with the idea that the lower degree of crowding in these lines may have increased the mean count. Crowding is known to depress the count (Rasmusson, 1952) but it is a little surprising that our control conditions, which we had considered optimum, should have such an effect. LR is below the average of the two controls in gen. 17A (0.67 ± 0.39) and the value of the differences in the generations 11–17 of the experiment suggests that this

difference may be real. HR does show a definite increase above the controls (2.77 ± 0.29) in gen. 17A. Our impression during the experiment was that this occurred in the early generations and the final figure was not as large as we expected earlier. Perhaps this is due to an isolated event rather than an accumulation of small effects.

The average variance of the two sexes calculated within generations is given in table 3. The two control lines have been averaged because of their similar behavior. The figures for each line are based on 48 d.f. per generation except for 17A where they are based on 98.

TABLE 3

Generation	Controls	HR	LR
1–5	5.03	6.39	5.72
6–10	4.60	7.55	5.44
11–17	4.43	8.91	4.62
17A	3.87	6.07	6.79

The trends are not as clear as they are for the means. The controls decline slightly but the effect is not significant. HR is more variable than the controls as might be expected from the response to selection. However, had all this extra variance been utilizable by selection, the response should have been greater. Our base outbred population has a mean variance of 12.2 and under this intensity of selection would respond at 1.5 chaetae per generation. LR is only slightly more variable than the controls except for generation 17A where the behavior of the two irradiated lines is surprising.

We had hoped to carry on selection for sterno-pleural chaetae simultaneously with this work but some of the lines were lost in the early generations. However, at generation 10 of LR, selection for sterno-pleurals was carried out for 5 generations in both directions under similar conditions to that described above for abdominal chaetae. There was no detectable divergence between the lines.

In experiments of this sort, it is essential to guard against the possibility of contamination. A response to selection may be due to a single contaminant. When this work was started no suitable inbred stocks were available in this laboratory though they have now been obtained. It was not realized until too late that our line was in fact marked. Examination of salivary chromosomes from LR at the end of the experiment, kindly made by Mr. G. R. Knight, showed that the line was homozygous for In(3R)K known to be present at low frequency in the base population. Unfortunately HR, the line with the greatest response, had by then been discarded. It may be noted that no new inversions were found in the 10 larvae examined in LR.

DISCUSSION

In mutation work on lethal or visible genes, the mutation rate can be simply stated as the proportion of chromosomes in each generation which gives rise to a change of a certain type. But for quantitative characters,

we have no such absolute measurement and no way of bringing the results for the new variation on to the same plane of reference as those for "good" genes. Presumably our measurement of the effect of radiation in producing new continuous variation would be in terms of the increase in variance for a given dosage. One is still faced with the problem of comparing the new variation arising in different characters. A possible standard of comparison might be the genetic variance of the character usually observed in wild populations. This is perhaps vague, particularly for domestic animals, but it does have the merit of giving a figure which is also of evolutionary interest, i.e. the problem of how long it would take for a genetically invariant population to acquire as much variation as is usually found in the wild, solely by the accumulation of mutations.

In the character we used, the few observations on wild populations of *Drosophila melanogaster* suggest fortunately that the greater part of the genetic variance is additive and can therefore be measured from the response to selection. The latter can then be expressed as the product of the heritability of the character concerned and the selection differential. The heritability is equal to $\frac{\sigma^2 g}{\sigma^2 p}$ the ratio of the additive genetic variance $\sigma^2 g$, to the phenotypic variance $\sigma^2 p$ and, on the assumption of normality, the selection differential can be expressed as $\bar{i}\sigma p$, where $\bar{i}$, depending on the degree of selection, can be obtained from tables (Fisher and Yates 1938, table 20). We then have for the average response in each generation

$$\Delta G = \frac{\sigma^2 g}{\sigma^2 p} \cdot \bar{i}\sigma p; = \bar{i}\,\frac{\sigma^2 g}{\sigma p}$$

For the two control lines, the last two entries show HC higher than LC by 0.06 ± 0.36 and 0.30 ± 0.64. We may combine these to give a mean value of 0.12 ± 0.32. As an upper limit for this divergence, we can take the mean plus twice the standard error, i.e. 0.76 bristles, so that the response in each line would be 0.38. This would occur in about 14 generations giving an average response each generation of not more than 0.027. In the controls, $\bar{i} = 0.94$ (corresponding to 10 selected out of 25) and $p = 2.1$ (table 3). We have then

$$0.027 = 0.94 \times \frac{\sigma^2 g}{2.1}; \text{ i.e. } \sigma^2 g = 0.060$$

This is an average value over the 14 generations. The line had been continuously mated full-sib before the start of this experiment and assuming that equilibrium had been reached between the loss of variance due to the mating system (at a rate of 19.1 per cent per generation) and new variation arising per generation by mutation we would have for $\sigma^2 g(o)$ the genetic variance present at the start where

$$0.191\ \sigma^2 g(o) = \sigma^2 gm$$

where σ^2gm is the amount of new variation produced each generation. Thus $\sigma^2g(o) = 5.2\sigma^2gm$. During the experiment, the relative loss of variability will be $\frac{1}{2N}$, where N is the number of parents, in this case 20. Thus at generation K: $\sigma^2g(K) = 0.975\sigma^2g(K-1) + \sigma^2gm$. This gives for the fourteenth generation $\sigma^2g = 15\sigma^2gm$ giving an average over the experiment of $10\sigma^2gm$. The upper limit to σ^2gm is thus 0.006 units per generation. In the few wild populations that have been examined, the genetic variance has been about 5 units. The rate of spontaneous production of new variance in each generation is thus probably less than about .001 of that present in wild populations.

Two experiments bearing on the spontaneous origin of variation in this character have been carried out by Mather and Wigan (1942) and by Durrant and Mather (1954) both using the same Oregon stock, full-sib mated for 78 generations by 1942 and for over 300 generations by 1954. Mass selection for abdominal and sterno-pleural chaetae was done in the first experiment. The intensity of selection was higher than in our work (the extreme 1 in 20, corresponding to an $\bar{i}$ of 1.87) and the mating system involved slight inbreeding which would cause a loss of variance of about 5 per cent per generation. One might expect that the genetic variance would gradually increase until it stabilized at around 20 σ^2gm. Progress was slow but definite in the first phase of the abdominal selection but increased in the later stages. The results in the last phase can be put into our framework as follows. The rate of divergence from generations 34-53 was roughly 0.2 bristles per generation, giving a rate of change in each line of 0.1. σp is given in table 3 of the paper as about 2.5. We have then

$$0.1 = 1.87 \times \frac{\sigma^2 g}{2.5}; \sigma^2 g = 0.13$$

If we assume this to be equal to 20 σ^2gm, we obtain a value for the latter of 0.007 units per generation, of the same magnitude as our upper limit.

In the second experiment, a sample of 10 second chromosomes was taken from the inbred line and all possible zygotic combinations were formed. Significant differences between chromosomes were found in the bristle counts of zygotes containing them and the component of variance between chromosomes was 0.056. The rate of production of new variance between chromosomes is thus, by the argument presented earlier a fifth of this, 0.011, as the line had been mated full-sib. The new variance between diploid individuals is a half of this giving a value of σ^2gm for the second chromosome alone of 0.006 units per generation, again of the same order of magnitude.

Selection of sterno-pleurals was only done for 21 generations, producing in that time a divergence of one chaeta. This gives a value of 0.020 for the average value of σ^2g and of 0.002 units per generation for σ^2gm. The chromosome assay gives 0.002 units for σ^2gm for the second chromosome

only. In our outbred population, the genetic variance in sterno-pleurals is 1.7 units.

The experiments of Mather and his co-workers both suggest that the rate of production of new variance by mutation each generation is of the order of .002 to .001 of that present in wild populations both for abdominal and sterno-pleural chaetae. If rates of this magnitude are found for other characters and in other animals, the effect of mutation may in general be regarded as negligible in selection experiments in laboratory animals and, even more so, in domestic animals where such experiments rarely include even as many as five generations of selection. But from the evolutionary point of view, this rate must be considered as high. Many recent investigations have revealed a surprising amount of variability latent in wild populations which has in turn led to speculation as to the mechanisms preserving this variability in the population. It would seem from these results that, for characters such as numbers of sterno-pleural or abdominal chaetae, which are probably in themselves selectively neutral, the existing variation could well be maintained by the equilibrium between inbreeding and mutation, without the necessity of invoking mechanisms involving selection. In the absence of selection, equilibrium between mutation and inbreeding will be reached when the genetic variation in the population is equal to $2N\sigma^2gm$ where N is Wright's effective population size. The variation found in our populations would thus only require that N should be of the order of hundreds. The only estimates of N for Drosophila, that of Wright, Dobzhansky and Hovanitz (1942) in *D. pseudoobscura*, were of the order of thousands or tens of thousands.

If we take the HR line, as that which showed the greatest response under selection, we find in generation 17*A* a deviation from the control means of 2.77 chaetae. Putting $\sigma p = 2.8$ in this case (table 2) we have $2.77/17 = 0.94 \times \sigma^2 g/2.8$ i.e. $\sigma^2 g = 0.49$. Taking this average value over all generations to be the actual value at the eighth generation, we get for the rate of accumulation of new variation a value of 0.06 units per generation or about 10 times the value calculated as the higher limit for spontaneous mutation. Thus at this rate it would need about 80 generations with a dose of 1800 r a generation to produce variance equal to that in wild populations.

However the observed increase in variance at the 6–10th generations was about 3 units compared with the calculation of the part utilizable by selection as 0.49. Perhaps the accumulation of lethals and steriles in the population may mask the utilization in selection of the new variation because many of the chromosomes will be unfixable, unless a crossover occurs at the right place. We propose to irradiate the random-bred base population and observe the effect on its utilizable variation. If this is important, it may be that other breeding programs, involving measures to reduce the frequency of recessive lethals, would be more successful than simple selection in making use of the new variation.

Apart from the experiments of Scossiroli and Buzzati-Traverso, referred to earlier, there are few other records of selection after irradiation. Lewis

(1949) reports work carried out by Harrison rather similar in design to our own. Males from an inbred line were given 4000 r each generation and selection was for abdominal chaeta number. Responses were obtained in the down lines but only in one of the up lines.

We are indebted to Dr. I. M. Lerner for drawing our attention to two papers by Russian workers who selected for sterno-pleural chaetae after irradiation. Serebrovsky (1935) started with a "Florida" stock of *Drosophila melanogaster*, which, judging by the variability at the start of the experiment, must have been inbred, though no mention is made of this. He gave 2000 r to males, 1000 r to females and used a mixture of family and individual selection with full-sib mating. After 8 generations the divergence between the control lines was greater than that between the irradiated lines. Using the four selected lines as starting material, he then changed to individual selection with much lower inbreeding. In the succeeding 10 generations, he obtained a divergence of 6 chaetae between the control lines and of only 2 between the irradiated lines. It is surprising that such variation should have remained in the control lines after 8 generations of full-sib matings.

Rokizky (1936), a pupil of Serebrovsky, then extended this work in more detail. In the first series he used the same Florida stock as Serebrovsky and a similar breeding and selection system as in the latter's first experiment. He only selected upwards with three lines, control, irradiated (3000 r in ♂) and temperature shocked (35–36°C in larvae). Selection for 12 generations produced little change in any of the lines. In his second series, starting this time from a Caucasus stock, he selected upwards 40 lines, 20 controls and 20 irradiated. Full-sib mating was carried out and selection was based on individual score for 25 generations. He again gave males 3000 r each generation. The results are summarized in table 4.

TABLE 4

Generation	Radiated		Control	
	Mean	S.D.	Mean	S.D.
1–5	20.01	2.11	19.80	1.96
20–25	20.61	2.63	19.96	2.17

The inbreeding system practiced severely reduces possible responses. The slight increase in the controls is not inconsistent with Mather's results. The radiated lines show a more definite increase and, as do our irradiated lines, an increase in variability. There was considerable heterogeneity of behavior amongst the irradiated lines. One gained 3.5 bristles with an average standard deviation in the later generations of 2.31 and another gained 1.4 bristles with an average increase in standard deviation to 3.26.

Our own results would suggest that the production of new variation in abdominal bristles by mutation is slow. Under irradiation, new variation can be detected but that utilizable by direct selection is small. The general tenor of the reports of similar experiments by other workers is in agreement,

with the exception of those of Scossiroli (1953). In the latter case, the disagreement may be due to the fact that we started from an inbred line and he used a line which had ceased to respond to selection. Some of our results are not completely explainable and we regard this only as a pilot experiment leading to more detailed work with suitably marked stocks on both spontaneous and induced variation.

SUMMARY

Selection for abdominal chaetae has been carried out in an inbred line of *D. melanogaster*, both with and without irradiation of 1800 r of X-rays each generation. The response in the control stocks in 17 generations was not significant. The irradiated lines responded to selection but slowly compared with wild populations.

This is discussed in relation to the results of other workers. Two papers by Mather and co-workers are found to give consistent estimates of the rate of spontaneous production of new variance in abdominal chaetae of the order of 0.01 units each generation, which is not inconsistent with our results. The variance found in several wild populations is about 5 units. The evolutionary aspect of these results is discussed.

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PHENOTYPIC (PHENOMIC) LAG IN BACTERIA*

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The commonly accepted interpretation of a variety of experiments with bacterial mutants is that after a mutation has occurred in a genetic factor some long time, measured in generations, is required before the phenotype is modified. Two main lines of evidence bear on this point. The first (but not historically), which concerns a discrepancy in rates of mutation obtained by different methods (Newcombe, 1948; Newcombe and Hawirko, 1949), has been shown elsewhere (Ryan, Fried and Schwartz, 1954) to be inconclusive. The second and most frequently invoked evidence for phenotypic lag is that the full complement of induced mutations is not expressed immediately after a mutagenic treatment (Gray and Tatum, 1944; Roepke, Libby and Small, 1944; Demerec, 1946). Rather, if a culture that has been exposed to the mutagen is allowed to undergo some growth, the frequency of mutants within it increases for a certain length of time (Demerec and Cahn, 1953; Labrum, 1953a and b). This time has been taken, at least in part, as that required for the expression of the mutant phenotype by bacteria that were genotypically mutated but still phenotypically like the parent. It is possible, however, that the delayed appearance of mutants may have many, not necessarily mutually exclusive, explanations (Witkin, 1951; Newcombe, 1953; Ryan, 1954).

The experiments that have been used most forcibly as evidence for phenotypic lag are those involving the selection of auxotrophic mutants by the use of penicillin (Davis, 1948, 1949; Lederberg and Zinder, 1948; Adelberg and Myers, 1953). In these cases it was shown that, when *Escherichia coli* was irradiated with ultraviolet light and directly suspended in medium devoid of growth factors but containing penicillin, no mutants could be recovered. On the other hand, when the irradiated bacteria were cultivated in a medium containing growth factors and then washed before resuspension in penicillin, induced mutants were readily found. It was assumed (Davis, 1950) that in the first instance the induced mutants were genotypically changed but phenotypically unmodified; hence, they grew in the minimal medium and were killed by the penicillin. The intermediate cultivation allowed the expression of the mutant phenotype and the failure of phenotypically mutant cells to grow in the minimal medium was supposed to have protected them from the lethal action of pencillin and allowed their concentration among the survivors. But the irradiated bacteria that were immediately

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subjected to penicillin were not washed and it is known that ultraviolet treatment causes the release of growth factors from the cells that are killed (Loofbourow, 1948). Thus, induced mutants that were expressed before growth occurred might utilize the released metabolites, be killed by the penicillin and, consequently, would not be found.

When wild-type *Escherichia coli* (strain 15) were irradiated with ultraviolet light to a survival of ca. 10^{-2} and introduced immediately into minimal medium containing 150 units of penicillin per ml., it was found that there was a frequency of 5.9×10^{-6} auxotrophic mutants among the bacteria surviving irradiation. This finding contradicts previously reported results. If the cells were washed once in saline after irradiation before immersion in penicillin, the frequency was 1.6×10^{-5}. Thus, some mutants appeared to be killed during their growth on factors released from cells killed by the ultraviolet light. Nonetheless, bacteria that were cultivated in nutrient broth for 12 generations after the mutagenic treatment, and then washed and subjected to penicillin showed an incidence of auxotrophic mutants of 3.6×10^{-4}. Mutants were identified by replication (Lederberg and Lederberg, 1952) followed by isolation and individual test. Because some death of the auxotrophic mutants does occur in penicillin, the number of bacteria introduced into the penicillin medium was equated in every experiment and the time in penicillin was adjusted so that the killing was the same. Consequently, although a period of growth intervening between the mutagenic and penicillin treatments was not required, intermediate cultivation increased the frequency of mutants.

The mutants appearing without intermediate cultivation could not be those existing in the culture before irradiation and whose phenotypes were already expressed. When unirradiated bacteria were subjected to penicillin treatment after washing, with or without intermediate cultivation in nutrient broth, a frequency of 1.2×10^{-6} mutants was found. Therefore, at least 90 per cent of the mutants studied in the previously mentioned experiments were induced and new.

If the genotypic mutants newly induced by irradiation were initially phenotypically wild type and underwent a residual growth of penicillin, *some* might have expressed their mutant phenotypes and stopped growing before they were killed by penicillin. The most straightforward explanation of the mutants appearing without cultivation is, however, that they resulted from mutations which did not involve a phenotypic delay of an detectable length. The increase in mutant frequency upon cultivation can also be explained without the *ad hoc* assumption of phenotypic lag. It is well known that most auxotrophic mutations are recessive (Lederberg, 1949) and that mutations in *E. coli* arise in one of the nuclei in a multinucleate cell (Witkin, 1951; Ryan, Fried and Schwartz, 1954). In this event, bacteria containing one mutant nucleus would grow in penicillin through the action of the unmutated sister nuclei in the heterocaryon and, as a consequence, the cell would be killed and the mutant nucleus lost. On the other hand, intermediate cultivation would allow for the segregation of the progeny of the mutant nucleus

into a homocaryotic cell which would be mutant, would not grow in penicillin and would survive. The mutants that appear without cultivation could derive from those rare heterocaryons which happen to survive penicillin during the few generations of growth required to give rise to a homocaryotic mutant. Or they might be due to the few mutations to auxotrophy that are dominant, or to recessive mutations in nuclei whose sisters were killed by the mutagenic treatment.

Still other explanations exist for the effect of intermediate cultivation such as delayed mutation or the selective overgrowth of at least one of the auxotrophic mutants during cultivation. In the present instance the mutant would have to grow about 20 per cent faster than its parents to account for the results. Were such the case, all of the auxotrophs recovered after cultivation would require the same growth factor; this was not tested in the present experiments.

There can be no doubt that some time *must* elapse between a genotypic and the consequent phenotypic change; the question at issue is how much time is required. In the case of mutations to auxotrophy, at the time of genotypic change, the parent cell is provided with enzymes that allow the synthesis of growth factors. Some of these may be inactivated by the mutagenic agent but we have no reason to believe that all are. The work of Cohn, Hogness and Monod (1955) indicates that in bacteria there is no detectable degradation of enzymes through molecular turnover. The delay between genotypic mutation and phenotypic change would then involve the dilution of the surviving enzymes to a sub-threshold level. McIlwain (1946) and Roper (1948) have shown that the number of enzyme molecules *necessary* for the observed rates of synthesis of growth factors by the bacterial cell are of the order of one. If these enzymes are not the genes themselves but *are* present in small numbers, division would be required for their dilution but the time required for their loss may not be large or even greater than that needed for the segregation of the mutant nuclei into a homokaryon.

Mutations from auxotrophy to phototrophy may or may not require the complete synthesis of growth factor-synthesizing enzymes. Davis (1950) has shown that induced mutants without a tryptophane requirement are more frequent in *E. coli* when there is an intermediate cultivation in tryptophane. This result can be explained in terms of the recessive nature of the mutation in a heterocaryon, with or without a specific delay in the onset of growth of the mutant (Ryan, 1954) or by delayed mutation, as well as by phenotypic lag. Similar results were not found with mutation to histidine non-requirement. Cultivation of freshly induced mutants in histidine did not increase their frequency and independent evidence indicated that phenotypic delay was undetectable and shorter than one generation (Ryan, 1954; Ryan, Fried and Schwartz, 1954).

SUMMARY

The purpose of this communication is to indicate that there is no evidence in the experiments referred to that compels the interpretation that the pheno-

typic expression of a genetic change requires a long time, or even one division, for its completion.

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A STATISTICAL EVALUATION OF THE KINSHIP OF PROTEIN MOLECULES*

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The literature contains many treatments of variation in amino acid composition of protein (for example, Bailey, 1950; Tristram, 1953). With the fuller development and application of amino acid assays there have appeared at least a few comments which particularly emphasize similarities in composition (Block and Bolling, 1951; Fox and Winitz, 1952; Tristram, 1953). Because of some differences in emphasis, an attempt has been made to evaluate the available data quantitatively. Parenthetically, it may be noted that the closely similar structural relationships of many molecules (Baldwin, 1948), such as vitamins E (Karrer and Fritzsche, 1938), penicillins (Clarke, 1949), and porphyrins (Granick, 1953) are not as readily if at all, reduced to statistical analysis (compare Stanley, 1947).

For the purpose indicated, the protein composition table published by Haurowitz (1950) has been employed. Ammonia was disregarded. Amino acids for which five or more estimations were missing were omitted from the statistical analysis. After this deletion, proteins for which three or more amino acid estimations were missing were also omitted. The sums of leucine and isoleucine and of cysteine and half-cystine were used inasmuch as assay figures for the individual amino acids of these pairs were not sufficiently available. Additionally, α-casein and β-casein were omitted inasmuch as their inclusion with casein would have influenced the evaluation in an unduly favorable way. After these omissions and combinations, the six remaining missing values, from a possible total of 384 values, were estimated by a least squares procedure (Snedecor, 1946) to facilitate the analysis. The amino acids thus considered were: arginine, histidine, lysine, glutamic acid, aspartic acid, glycine, valine, leucine and isoleucine, proline, phenylalanine, serine, threonine, tyrosine, tryptophan, cysteine and half-cystine, and methionine. A total of twenty-four proteins was included in this analysis. An analysis of variance of the following type was computed (table 1).

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TABLE 1

MODEL FOR ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Sum of squares	Expected mean square
Amino acids	$a-1$	$p\sum_i (X_{i.} - X_{..})^2$	$\sigma_P^2 + p\sigma_A^2$
Proteins/amino acids	$(a-1)(p-1)$	$\sum_i \sum_j (X_{ij} - X_{i.})^2$	σ_P^2

In this analysis,
X_{ij} = the amino acid content for the j-th protein and the i-th amino acid,
$X_{i.}$ = the mean for the i-th amino acid,
$X_{..}$ = the mean of all observations,
a = the number of amino acids, and
p = the number of proteins.

From this analysis an index of correlation can be computed as follows:

$$I = \frac{\sigma_A^2}{\sigma_P^2 + \sigma_A^2}.$$

This index is 1.000 when σ_P^2 is 0.000 which will occur when the amino acid composition of all proteins is the same. The index is 0.000 when σ_A^2 is 0.000 which will occur when the variation among proteins within amino acids is equal to that among amino acids. In other words, the index would be 0.000 if the amino acid values were distributed at random to the proteins. The index is negative when the variation among proteins within amino acids is larger than that among amino acids. This would indicate more variation in the amino acid composition among proteins than could be attributed to random variation. The largest possible negative value of the index is $-\frac{1}{p-1}$

The above analysis of the data in Haurowitz' table resulted in an index of correlation equal to 0.487. The sum of squares for proteins is combined with that for the interaction of proteins with amino acids. If the determinations of all the amino acids for the proteins being considered were available, then the averages of the amino acid residues for each protein would be essentially the same and the variance among them would be near 0.000. If the variance among proteins were actually 0.000, a slightly different analysis would have been used. The variation in amino acid contents among proteins within amino acids was given $(a-1)(p-1)$ degrees of freedom instead of $a(p-1)$ because the same proteins are represented for each amino acid.

It seemed desirable to perform a similar analysis on proteins of one type in closely related organisms in contrast to the wide spectrum in the Haurowitz table. After this need was recognized the first table which came to hand was that involving globulins from seeds of different plants (Smith and Greene, 1947). The amino acid values in this table that are also included in the Haurowitz table and were not omitted in the present analysis are arginine, histidine, lysine, threonine, leucine plus isoleucine, valine,

tyrosine, tryptophan, phenylalanine, and methionine. An analysis of the values for these amino acids, by numbers of residue of each type, gave an index of 0.966. The index for the Haurowitz table was recalculated for only those amino acids listed above. This gave an index of 0.625 (table 2).

TABLE 2

SUMMARY OF INDICES OF CORRELATION

Relatively widely varied proteins (Haurowitz, 1950)	Index*
16 amino acids	0.487
10 common amino acids	0.625
Seed globulins (Smith and Greene, 1947)	
12 amino acids	0.966
10 common amino acids	0.973

*An index of 0.000 would indicate that the amino acid composition among proteins is independent or unrelated, and an index of 1.000 would indicate identical composition of all proteins being considered.

Each of the results of Table 2 conforms with the explanation that all proteins are molecular descendants (Fox, 1953) of a common primordial ancestor (Fox and Middlebrook, 1954; Fox, Johnson, and Middlebrook, 1955). Although physical factors may interfere internally with formation of some of the otherwise conceivable residue arrangements in protein (Fox, Winitz, and Pettinga, 1953), protein synthesis cannot be said to be essentially limited. Rather protein synthesis has not, in the main, yet become sufficiently diverse through molecular evolution to yield substantially unrelated proteins. An almost infinite number of unqualifiedly theoretical structural arrangements of amino acid residues can be suggested (Asimov, 1954). So far, in the course of one or two billion years, there has evolved only a minute fraction of the structural types which theoretically may result from metabolism operating in its probably limiting intramolecular framework. Furthermore, neither the primordial nor subsequent syntheses (although sufficiently replicable to permit inheritance) should be considered to be either fixed or limited (compare Fox, 1953; Atkinson, Melvin, and Fox, 1951 and Baker and Fox, 1954; Levine and Tarver, 1951, Haldane, 1954; and Huxley, 1954).

A low rate of molecular evolution, probably much lower than the rate of biological evolution, is implied by this interpretation. Although diversity discernible in organisms is, to our eyes, many orders of magnitude greater than a statistically correlative current similarity in compositions of proteins, no special difficulty is posed by this comparison. If all the molecules which compose an organism are taken into account, the diversity in organisms should be an exponential function of diversities in molecular synthesis. In the same general way that a huge number of proteins may arise, theoretically or actually, from twenty-odd amino acids, an exponentially tremendous number of individual organisms could result from the interactions of perhaps a few dozen or few hundred proteins and other biochemical substances.

The table in Haurowitz' book appeared to be especially adapted to the primary purpose of the present statistical analysis inasmuch as it contains a wide variety of proteins by type and source, and relatively complete analyses by numbers of residues. A major fraction of the proteins is nevertheless from vertebrate or mammalian sources. In view, however, of the interpretation discussed here and the evolutionary lateness of mammalian forms, a statistical establishment of kinship appears to be strengthened by this kind of disproportionateness.

In the light of these considerations, it is not surprising that the proteins which most lowered the index were such as collagen. The collagens, important for connective tissue, would not have been of selective advantage to evolving organisms until upper levels of phylogeny were attained. This fact thus correlates with their greater departure from a presumed primordial composition.

Inasmuch as protein composition is a function of residue arrangement it is to be expected that these considerations may be extrapolated to protein structure. Some evidence of this sort, presented without such interpretation, is at hand (Fox, 1945; Porter and Sanger, 1948). Additionally, analyses of seeds of wheat, rye, and corn for ten amino acids in the terminal position in the protein mixture have revealed only lysine in substantial proportion, with arginine outstandingly penultimate of six amino acids analyzed (Fox and De Fontaine, 1954). Similarly three proteins separated from chicken egg white each contain N-alanine (Fraenkel-Conrat and Porter, 1952). A high degree of structural homogeneity in the overall protein makeup of individual organisms is thus suggested.

Recognition of the kinship of protein structures by virtue of a finite evolution from a primordial ancester has a number of other consequences. For example, the probability that two terminal pentapeptide residues in an active and an inactive fraction of γ-globulin will be identical is many orders of magnitude greater than 1 in 18^5 (Porter, 1950) inasmuch as sequences in different proteins are not independent of one another. The significance of structural similarity between two protein molecules of similar source but different biological activity is thus more questionable than otherwise.

Another consequence of the evolutionary interpretation makes contact with the many past efforts of protein chemists to find some regularity in protein structure. In a molecular evolution involving essentially small changes in composition, and therefore structure, a search for regular chemical periodicity in all proteins would be recognized as doomed to failure. The regularity now suggested is not within molecules but between molecules. This intermolecular regularity of close kinship would involve relatively small, probably stepwise, differences in structure.

It will be of interest to see if statistical evaluation of correlations in amino acid composition may be of value in filling gaps in knowledge of biological evolution, by locating or confirming close evolutionary relatives of e.g. collagen (connective tissue), myosin (muscles) oxytocin (pituitary gland) (note similarity of oxytocin and vasopressin, Du Vigneaud, Lawler, and Popenoe, 1953), or γ-globulin (antibody synthesis).

These arithmetic results and interpretations do not deny the complexity of existent biochemistry. They do suggest, however, a discernibly high degree of kinship between protein molecules, or a complexity of types which is not as hopelessly incomprehensible as has often been presupposed. When viewed in the light of current experimental study (Fox, Johnson, and Middlebrook, 1955) it becomes possible to visualize in outline how this degree of complexity might have stemmed from relatively very simple beginnings.

SUMMARY

An index of correlation suitable for comparison of protein compositions is constructed. The index is defined and its limits stated. Upon application to six seed globulins, as evaluated for ten amino acids, the index is 0.973. For the same ten amino acids in twenty-four proteins of varied type and source the index is 0.625.

These results indicate a high degree of kinship for "heterologous" proteins and a higher degree of kinship for "homologous" proteins. The significance of this fact in relation to theoretically possible structures of protein, variability in synthesis, qualitative considerations of rate of molecular evolution, actual protein phylogeny, structural regularity between protein molecules; and related concepts, is discussed.

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TECHNIQUES FOR STUDIES OF HEMOGLOBIN SYNTHESIS IN DAPHNIA

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In recent years H. Munro Fox and colleagues (1951, 1953) have made extensive studies of the functions of hemoglobin and factors influencing hemoglobin synthesis in Daphnia. In these studies the hemoglobin concentration in the blood of living Daphnia was measured by an index method in which the color of a known dilution of laked human blood is matched with the blood colors of 10 or more individual Daphnia alined out of water on a microscope slide. The mean of the hemoglobin values, each read on a scale, gives a hemoglobin index of the population. The method is dependent upon the color matching ability of the observer and is inadequate to determine accurately the hemoglobin content of pale animals. A spectrochemical method for measurement of total hemoglobin content in experimental populations of *Daphnia magna* has been devised in this laboratory.

MATERIAL AND METHOD

A modification of the original cyanhemoglobin method for mammalian blood devised by Drabkin and Austin (1932) is utilized. Animals from a culture flask are netted and transferred to a large watch glass containing distilled water. This serves to wash the animals free of culture medium. The water is then removed with a fine pipette, and 25 adults are carefully picked up five at a time with a small, flat spatula, gently blotted with wiping tissue to remove adhering water, and transferred to a 1 ml. centrifuge tube containing 0.20 ml. of cold distilled water. After a series of cultures or aliquots of Daphnia are prepared in this way, the animals are macerated with a glass rod which fits closely into the bottom of the centrifuge tube. The tissue debris is quickly packed down again, and preparations are centrifuged for about five minutes in a clinical centrifuge. All of the pale-red supernatant is removed with a fine pipette and transferred to a 10 mm. × 75 mm. tube. Then 0.03 ml. of 0.1% $K_3Fe(CN)_6$ is added and allowed to stand for 10 minutes. Finally 0.02 ml. of 0.1% KCN is added to convert the methemoglobin to cyanhemoglobin.

The cyanide method has the advantage of inactivating the enzymes derived from the macerated animals. A very slight turbidity is present in the final preparations. This does not affect the hemoglobin determination, but it may nonetheless be cleared by the addition of one drop of concentrated ammonium hydroxide. The final preparations (0.25 ml.) are placed in 6 mm.

O.D. cuvettes which fit the No. 6–110 semi-micro adapter for use with the Coleman 6A Junior Spectrophotometer. In practice, the instrument is adjusted to 100% T with a reagent blank (0.20 ml. H_2O, 0.03 ml. 0.1% $K_3Fe(CN)_6$, 0.02 ml. 0.1% KCN).

RESULTS

Spectral-Transmittance curves of Daphnia cyanhemoglobin are shown in Figure 1. Distinct minima are obtained at 415 mu, and this is characteristic

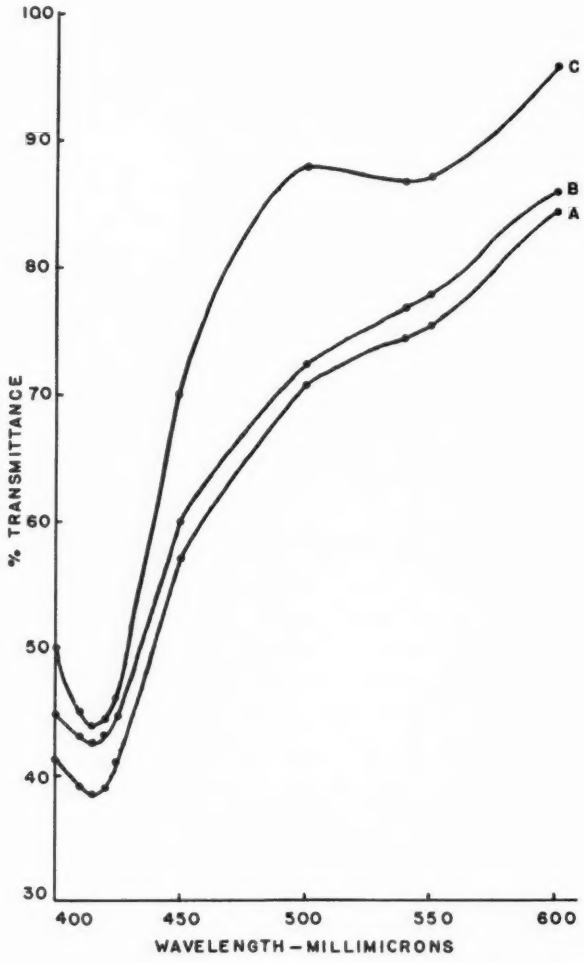

FIGURE 1. A, B. Spectral-Transmittance curves of cyanhemoglobin from two samples of 25 Daphnia each, from the same culture. C. Spectral-Transmittance curve of rabbit cyanhemoglobin standard.

of all Daphnia preparations from quite pale to dark red animals. Maximal light absorption in the 415 mu region is known to be specific for hemoglobin. The curves plotted are those of two aliquots of 25 adult Daphnia each, taken from the same culture at the same time. The Spectral-Transmittance curve of an identical volume of rabbit cyanhemoglobin of known hemoglobin concentration and with the same concentration of reagents is also shown. While the curves for the two species differ in detail, both show similar absorption maxima in the 415 mu region. The difference of 4% T between the two Daphnia samples at 415 mu has been the maximum difference observed between groups grown under identical conditions when the animals are selected for similar size and number of parthenogenetic eggs. Duplicate readings made on samples over a six hour period gave a maximal variation of only one per cent transmittance, indicating that the cyanhemoglobin preparations as well as the spectrophotometer were stable. Several Daphnia cyanhemoglobin preparations were diluted to various concentrations with water. The shape of the S-T curves over the 400–425 mu region was unaffected. Plots of per cent transmittance at 415 mu against dilution factors showed a straight line relationship in each instance, indicating that other soluble constituents were not interfering with the determination. Transmittance measurements of *D. magna* hemoglobin at this wavelength may therefore be made in conformity with the Lambert-Beer Law. Such measurements may then be converted approximately into milligrams of hemoglobin by reference to a Concentration-Transmittance graph for a rabbit cyanhemoglobin solution of known hemoglobin content measured at 415 mu, and plotted on semi-log coordinates. Thus for the reddest sample of 25 Daphnia observed in this investigation, 10% T is equivalent to 0.23 mg. Hb and for the palest sample of 25 Daphnia 67% T approximates 0.04 mg. Hb. It is apparent that at least a six-fold difference in hemoglobin content may be found in *D. magna* cultured under various conditions.

Fox et al. (1951) have shown by the hemoglobin index method that a manifold increase in the hemoglobin content of the blood in Daphnia results from oxygen deficiency in the water; pale animals become red. We have been able to verify this stimulative effect of low oxygen levels under a variety of conditions using the cyanhemoglobin method. A typical experiment will be cited.

Four 500 ml. Erlenmeyer flasks were prepared, each with 200 ml. of culture water and 40 adult Daphnia from the same stock culture. One flask was left open to the atmosphere as a control. Each of the other flasks was covered with an air tight rubber cap, sealed with paraffin wax, and the remaining air space flushed with tank nitrogen under a 2-pound pressure for one minute through two No. 22 hypodermic needles. Each flask was given air access via a No. 16, 20, and 27 needle respectively, and maintained at room temperature (21°C.–24°C.) for five days. The size of the needle determines the oxygen level in the flasks under these conditions. The hypodermic needles also maintain atmospheric pressure by allowing the carbon dioxide formed in respiration to escape. Dissolved oxygen levels were de-

termined daily by the rapid, microcolorimetric method of Loomis (1954), and were found to remain relatively constant during the period of the experiment. The results are summarized in table 1. While flasks 1 and 2 show no sig-

TABLE 1

HEMOGLOBIN CONTENT IN RELATION TO OXYGEN SATURATION

Flask	Oxygen Level[γ]	Hb Content of 25 Daphnia
1. Control	76% sat. (6.6 mg. O_2 per liter)	44.5% T (.08 mg. Hb)
2. 16 G needle	46% sat.	41.9% T (.09 mg. Hb)
3. 20 G needle	35% sat.	34.3% T (.11 mg. Hb)
4. 27 G needle	5% sat. (0.5 mg. O_2 per liter)	28.5% T (.13 mg. Hb)

[γ]Rawson's nomogram may be conveniently used for transforming oxygen values from one kind of unit to another. (Cf. Welch, 1948.)

nificant difference in terms of the experimental error (4% T), the animals in flasks 3 and 4 responded to low oxygen by a considerable increase in hemoglobin. The difference in hemoglobin content between the extremes is readily apparent to the naked eye in the living cultures. However, our preliminary experiments indicate that a week or more may be required for maximal hemoglobin synthesis by *D. magna* in response to low oxygen. The absorbance values given in Table 1 do not therefore represent the final hemoglobin contents attainable at the oxygen levels given. The oxygen level of 5 per cent saturation we have found to approach the lower limit at which *D. magna* may survive for an extended period at room temperature.

While recent investigators have been using controlled suspensions of algae such as Chlorococcum (Frank, 1952) and Chlorella (Fox and Phear, 1953) as food for Cladocera with excellent results, these have certain disadvantages in physiological studies. Such Daphnia cultures must be kept in the dark to avoid algal photosynthesis and must be stirred daily to bring the algae back into suspension. Incidental to this investigation, we have developed a semisynthetic food source for Daphnia as follows:

Distilled Water	1577 ml.
Versene Fe-3 specific*	23 ml.
Versene acid (EDTA) powder	0.1%
Ferric citrate powder	0.3%
KH_2PO_4	0.1%
Na_2HPO_4	0.1%
$Mg\ Cl_2$	0.1%
Bacto-Casitone (pH 7.1)	0.5%
Difco proteose-peptone	1.0%
Yeast extract	0.1%
Dextrose	0.5%

*The quantity used is based on Bersworth Chemical Co. Tech. Bull. No. 2 data indicating that 1 ml. Versene Fe-3 specific complexes 0.164 gms. Fe-3 at pH 8.

The ferric citrate should be dissolved in the solution of versenes in water to avoid ferric hydroxide precipitation. The other constituents may then be added in any order. The medium is autoclaved and stored in the cold. It has a pH of 7.7, a value which is quite satisfactory for *D. magna*. The versene acid is added to lower the final pH and to provide some sequestering action for magnesium and cobalt which may be limiting in hemoglobin synthesis. The use of versenes as non-metabolizable solubilizing complex-formers in culture media is described by Hutner et al. (1950). Ferric citrate is used in relatively high concentration, since the efficacy of this salt in Daphnia hemoglobin synthesis has previously been shown by Fox and Phear (1953).

The medium as given represents only a first approximation toward a satisfactory food source. In practice, 5 ml. of medium is added to each 10 liter battery jar culture every second day. Bacterial proliferation may be excessive unless there are some 200–500 animals present. Stock cultures have also been maintained in large tanks in this manner for several months. Experimental cultures have been generally set up in 500 ml. flasks with 240 ml. of water and 60 Daphnia, or one animal to 4 ml. of water. About 0.2 ml. of penicillin G sodium (Merck) at a concentration of 15 mg. per ml. may be added daily to each test culture to minimize bacteria. In this way greater quantities of media or substances being assayed for hematopoietic effects may be added without having bacteria reduce the dissolved oxygen levels. Penicillin appears to have no adverse effects on the animals.

No attempt was made to grow the Daphnia in germfree culture, although this has been done by others with a few animals for short periods (Stuart et al., 1931). If *D. magna* cannot reproduce in this medium in the complete absence of bacteria, then the protein and carbohydrate constituents may be primarily a food source for bacteria on which the Daphnia feed. In any event a single reproducible medium of this kind, embodying both organic and inorganic constituents in a buffered solution, is to be preferred over the various manure, soil, and cereal infusions generally used. Even when algal suspensions are employed, chelated metallic ions may have to be added, and one must contend with both algal and bacterial metabolism. The extent to which particulate, colloidal, and soluble constituents may be utilized or required in the diet of Cladocera is still an open question. Earlier work on Cladoceran food requirements (Stuart et al., 1931; Gellis and Clarke, 1935) is inconclusive, since the test media used were inadequate in the light of present knowledge.

Daphnia magna appears to be well suited for studies of hemoglobin synthesis. With a quantitative method of Daphnia hemoglobin determination now available, this species shows promise for assay of possible hematopoietic substances.

SUMMARY

A new spectrochemical technique for determination of hemoglobin content in pale as well as red Daphnia has been presented. By use of this method

the primary stimulus for hemoglobin synthesis of low dissolved oxygen content in the culture water has been confirmed. Spectral-Transmittance curves of Daphnia hemoglobin are figured and compared to those of rabbit hemoglobin. A method for controlling the oxygen level in culture flasks by the use of hypodermic needles and air tight rubber caps is described. A first approximation toward a semisynthetic medium for Daphnia is discussed.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

PHAGE PRODUCTION BY CROWN-GALL BACTERIA AND THE FORMATION OF PLANT TUMORS*

Recent investigations have shown that crown gall tumors can be induced by cell-free preparations from the crown-gall bacterium.[1] The possibility exists that this activity as well as the induction of tumors by *Agrobacterium tumefaciens* is due to a temperate phage. Experiments were designed, therefore, to determine whether a virulent strain (B6) of *A. tumefaciens* was lysogenic. Cultures in the logarithmic phase of growth (ca. 10^8 bacteria per ml.) were exposed to ultraviolet light and their density followed with a colorimeter. A characteristic residual growth succeeded by lysis was observed. The supernatant was assayed on a strain of B6 secured after extraordinarily heavy irradiation with ultraviolet light (ca. 10^{-5} survival). This strain was sensitive to the phage produced during the lysis of strain B6, and plaques were formed indicating a maximum titer in the ultraviolet-produced lysates of ca. 10^{10} phage per ml. The lysing material in the plaques can be multiplied by inoculation into a liquid culture of the sensitive strain. These phages do not infect the strains of *Escherichia coli* tested, nor does the sensitive strain of *A. tumefaciens* support the growth of coliphages (T_1 – T_7 and lambda). This *omega* phage from *A. tumefaciens* is present in lower concentrations in non-irradiated cultures of strain B6. The sensitive strain after treatment with ultraviolet light does not produce an infective agent against itself.

Preliminary experiments indicate that supernatants of ultraviolet lysed *A. tumefaciens* cultures when sterile-filtered and applied to carrot phloem with 10^{-7} M indole-3-acetic acid are capable of inducing proliferation not shown in controls. Whether or not these overgrowths are crown-gall tumors and are induced by the phage present in the filtrates is now under investigation. It is intended that other problems related to the role of the omega phage in transforming a normal plant cell to a tumor cell will also be investigated.

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THE SO-CALLED ELECTRIC ORGANS OF OSTRACODERMS

More or less general acceptance has been accorded the opinion advanced by Stensiö[4] that certain structures of the cephalic region of many ostracoderms were electric organs (or "electric fields"). Under critical examination, however, it becomes evident that this hypothesis is untenable. Westoll (1945) has criticized this belief on morphological grounds. To this should be added certain criticisms which may be advanced on ecological and physiological grounds.

An ecological correlation with reference to electric organs has been indicated by the work of Coates et al.[2] In fresh-water fishes such as the electric eel, *Electrophorus electricus*, the electric polarity is longitudinal; i.e., there are relatively few long series of electroplax units so that there is a high e.m.f. and a high internal resistance. Contrasted with this situation is the condition in marine forms, such as the electric rays (Torpedo, Narcine, etc.). In these the body is flattened and the polarity is dorso-ventral; that is, there are relatively larger numbers of shorter series of electroplax units, so that both the e.m.f. and internal resistance are comparatively low. Both of these arrangements are adaptive in that they allow the maximum deliverance of power from a given number of electroplax units by matching the e.m.f. and internal resistance of the organ to the higher electrical resistance of fresh waters or the lower resistance of marine waters.

These authors attempt to add examples of this correlation by citing the resemblance of the "electric organs" of the ostracoderms to those of the electric rays. They add (p. 22) that the ostracoderms "were supposedly marine in habitat", as are the electric rays. However, the virtually (if not totally) complete agreement that the ostracoderms were fresh-water forms invalidates this comparison, and makes these ostracoderm organs ecologically anomalous if they are to be considered electric organs.

Even more damaging to this belief is the correlation found between the nature of the integument and the possession of electric organs. Those fishes possessing such organs generally have small, almost vestigial scales (as in the Mormyridae)[3] or more usually show the total absence of even vestigial scales (as in the electric catfish, Malapterurus, the electric eel and the electric rays.[1,3]

Bone or the bone-like material composing scales or other dermal armor are, of course, rather good electrical insulators, hence exceedingly poor conductors. In the ostracoderms the organs in question were located between the solid bony endocranium ventrally and an overlying dorsal exoskeletal mosaic of bony plates.[4] These bony plates, according to Stensiö (p. 239), "consist of basal, middle and external layers, exactly as the other parts of the exoskeleton of the cephalic shield". There is thus presented the hypothesis that a large group of animals possessed electric organs which were completely surrounded by a heavy layer of good insulating material with no electrical outlet, certainly a physiologically unsound interpretation.

An examination of Stensiö's work, furthermore, indicates that the hypothesis under question was supported by evidence of the most tenuous nature (p. 243): "The possibility is not quite out of question of course, that the organs described here as electric fields might have been sensory organs with a special function. But since it is very difficult to understand what this special function could have been, and since the organs in question undoubtedly have certain superficial resemblances to electric organs, especially to those in Torpedo..., I feel inclined to think that in reality they were electric organs, and because of that they have been referred to as electric fields throughout this work."

In view of the meager evidence supporting it and the difficulties its acceptance would create, it would seem advisable that this hypothesis be dismissed. As to the nature of these organs, Westoll[5] (p. 345) suggests that they may have been sensory, "probably receptors of vibratory stimuli". Although his evidence is not too convincing, it at least avoids raising such difficulties as does Stensiö's hypothesis. At any rate we may well agree with Westoll that the non-committal terms "lateral and dorsal fields" be used and the term "electric fields" be discarded.

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BROTHER LAWRENCE CORY, F.S.C.

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SENSITIVE PERIODS AND APPARENT FRACTIONATION EFFECTS IN IRRADIATED DROSOPHILA

In *Drosophila melanogaster*, fractionation of a given dose of X-rays, or variation in the intensity of the radiation, have generally been found to be ineffective.[1,2] However the demonstration[3,4] of the existence of sensitive periods towards the mutagenic effect of X-rays suggests that if in a fractionation experiment the time intervals between successive treatments are such as to permit, as a result of the normal course of maturation, a considerable change in the proportion of immature to mature sperm, a spurious fractionation effect could be produced. The following experiment illustrates one form which this effect can take.

In the fractionated series, newly emerged Canton S males received four separate doses of 700 r delivered at 24 hour intervals to give a total dose of 2,800 r. In the non-fractionated control series, newly emerged males were kept for 72 hours before irradiation. At the time that the first series received the final dose of 700 r, the control series received an unfractionated dose of 2,800 r. Irradiation was carried out in air at 17°C at a dose rate of 738 r per minute (76 KVP, 6 mA, HVL 0.85 mm. aluminium, effective wave length 0.6 AU).

Each irradiated male was mated to three *Muller-5*; *bw*; *st* females and was transferred to fresh virgin females at intervals of 72 hours to yield five successive broods. Furthermore, the inseminated females were also shaken across to fresh culture tubes six days after the males had been removed. Thus for each brood there was a first and a second laying, the latter being derived from eggs fertilised by sperm that had been stored for 6 to 9 days in the female spermathecae.

Small mass cultures of the F 1 material were kept for one week in half pint milk bottles containing unyeasted honey-agar. After this ageing period, virtually all of the females had been inseminated. They were then cultured individually for the detection of sex-linked, recessive lethals. The F 1 males were back-crossed individually to *bw*; *st* virgin females for the detection of 2:3 translocations. Cultures were all kept at 25°C, except the F 2 cultures for the lethals, which were kept at 20°C. The difference was solely to make best use of available space in the constant temperature rooms.

In table 1, the data from the five broods are all pooled to enable a comparison to be made of the incidence of lethals and translocations in the

TABLE 1

ABSENCE OF "HEALING" IN SPERM STORED IN FEMALE SPERMATHECAE

Laying	Sex-linked Lethals		2:3 Translocations	
	Non-Fractionated Dose	Fractionated Dose	Non-Fractionated	Fractionated
First	91/1695 = 5.37%	80/1503 = 5.32%	33/1007 = 3.28%	39/901 = 4.32%
Second	59/1164 = 5.07%	39/833 = 4.67%	20/530 = 3.77%	18/507 = 3.55%

first and second layings respectively. The slight differences are not statistically significant, indicating the absence of any tendency for healing to occur while the irradiated sperm are in the spermathecae. This, of course, merely confirms the observations of other investigators, notably Muller.[5]

TABLE 2

BROOD DISTRIBUTION OF LETHALS AND TRANSLOCATIONS

Brood	Sex-Linked Lethals		2:3 Translocations	
	Non-Fractionated	Fractionated	Non-Fractionated	Fractionated
First	20/624 = 3.20%	39/435 = 8.96%	8/405 = 1.98%	20/338 = 5.91%
Second	59/666 = 8.86%	39/415 = 9.39%	21/372 = 5.64%	11/250 = 4.39%
Third	38/482 = 7.87%	36/409 = 8.79%	18/294 = 6.12%	24/276 = 8.69%
Fourth	27/534 = 5.06%	2/546 = 0.37%	5/243 = 2.06%	1/255 = 0.39%
Fifth	6/553 = 1.08%	3/531 = 0.56%	1/223 = 0.45%	1/289 = 0.35%
Total	150/2859 = 5.24%	119/2336 = 5.09%	53/1537 = 3.45%	57/1408 = 4.05%

In table 2, the figures from the first and second layings have been pooled and the data presented in terms of the successive matings. Considering the non-fractionated series first, the incidence of both lethals and translocations is in agreement with Auerbach's claim that the greatest yield is to be found in the second and third broods, indicating the existence of a sensitive period prior to the fully matured sperm.

Comparing now the fractionated and non-fractionated series, although there are no significant overall differences revealed by the grand totals for the two series, the administration of a fractionated dose altered the distribution of lethals and translocations within the successive broods, so that the peak at the second and third broods is no longer so marked. The alteration is presumably a reflection of a gradual change in the proportion of spermatids to spermatozoa during the 72 hours covered by the administration of the fractionated dose.

There is also a considerable difference between the later broods of the two series, both translocations and lethals being significantly fewer in the fourth and fifth broods of the fractionated series. If these broods are to be regarded as being derived from sperm that were still at the gonial stage at the time of treatment, the difference might be the result of some kind of intensity effect. Perhaps at a dose rate of only 738 r per minute, exposure for less than a minute at a time is insufficient to enable a very high concentration of mutagenic substances to accumulate, whereas continuous irradiation for nearly four minutes may achieve this. The recent observations of Haas[6] and his colleagues have demonstrated that, at higher levels at least, the intensity factor may be of considerable importance in *Drosophila*, while Alexander[7] has demonstrated that spermatogonial cells are considerably less sensitive towards X-rays than are matured sperm.

The slight fall in the incidence of translocations in the second brood of the fractionated series is not statistically significant, so whether or not

there is a tendency for reunion to occur at a stage just prior to the fully matured sperm is therefore still an open question.

Grateful acknowledgment is made to Mrs. E. G. Clark, Miss M. Gunson and Mr. J. Thomson for technical assistance, and to Mr. J. F. Richardson of the Commonwealth X-Ray & Radium Laboratory for carrying out the irradiation.

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BOOK REVIEW

THE GENETICS OF DYNAMIC EQUILIBRIA

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How do individual organisms and their aggregates in populations acquire and maintain the particulate genetic properties which provide those internal physiological equilibria best designed for successful existence in ever changing and often inimical surroundings? To this many-faceted question I. Michael Lerner devotes a brilliant discussion in his "Genetic Homeostasis."*

The main theses of the book may be condensed from the author's own opening statement. They are as follows: 1. The evolution of cross-fertilized organisms has created genotypes that produce developmental patterns with a considerable degree of self-regulation. 2. Mendelian populations also possess self-equilibrating properties for the preservation of genotypes with maximum fitness in particular environments. 3. Superiority of heterozygotes over homozygotes is the most likely mechanism for attaining these ends by ontogenetic self-regulation ("developmental homeostasis") and by self-regulation of populations ("genetic homeostasis," i.e. natural selection favoring intermediate rather than extreme phenotypes). 4. It is, therefore, postulated that levels of obligate heterozygosity exist in Mendelian populations which benefit individuals by limiting deviation from the phenotypic optimum for fitness in populations by avoiding excessive reproductive waste and by better adjustment to environmental fluctuations. 5. It is suggested that the sporadic occurrence of morphological abnormalities ("phenodeviants") is caused "by the intrinsic properties of multigenic Mendelian inheritance, due to which a certain percentage of individuals of every generation falls below the threshold of the obligate proportion of loci needed in a heterozygous state to ensure normal development," and that these events comprise the "biology of inbreeding degeneration." 6. Additively acting polygenic systems are assumed to account for the inheritance of metric traits and the totality of traits determining reproductive capacity (fitness) to exhibit overdominance. 7. Finally, it is suggested that "attempts to shift populations too rapidly and too far from adapted mean values for specific traits, either by artificial selection or by changes in the breeding system, are counteracted by natural selection which is directed toward the maintenance of a phenotypic balance between fitness-determining characters" and that this resistance "can be overcome when new balanced combinations based on the utilization of free genetic variability arise."

*I. M. Lerner, Genetic homeostasis, New York (Wiley), 1954. VII, 134 pp. 14 figs.

These are the propositions which in the remainder of the book are expanded and for which such proof is adduced as the literature and the author's own investigations afford. They are, as the author himself well recognizes, no more than working hypotheses, offered as a stimulus to future study and thought. As such they deserve the close attention of all who are concerned with problems of evolution, developmental genetics and breeding for traits of economic value.

One may perhaps express some misgivings about the application of the term homeostasis, to which Cannon has given such concrete physiological meaning, to problems—and at that to two probably related, but certainly not identical sets of problems—of which the basic causal mechanisms remain obscure and debatable. For, homeostasis as the physiologist understands it, is a process of internal readjustment, whereas the equilibrating processes here at issue are consequences given by (not responding to) selective forces which expiscate particular genotypes and reject others.

The idée maîtresse of Lerner's book, viz. the importance of heterozygosity for success, on the individual and the population level, finds, of course, much support in observations on inbreeding and heterosis. Yet, heterosis is not an invariable consequence of the crossing of inbred lines. There is indisputable evidence that heterozygosity may at times play an important role in the maintenance of wild populations. Yet, this is certainly not invariably true. In the reviewer's own experience with fowl, homozygosity for recessive modifying genes is a common and effective mechanism by which disadvantageous mutations are completely submerged. The wide distribution of such modifiers and their action on very diverse mutants attest to their importance. Two examples may serve to illustrate this point. The defective plumage of Frizzle fowl is modified toward the normal condition by a single pair of recessive genes; more normal functioning of the thyroid gland of the same mutant form is accomplished by multiple recessive genes; both kinds of modifiers counteract the metabolic handicaps imposed by the Frizzle mutation; both kinds seem to be a common part of the gene inventory of normal breeds of fowl.

The emergence of mutant types has been repeatedly observed in fowl subsequent to and as a consequence of outcrossing. A recent (unpublished) observation of this kind is particularly illuminating. Cyclopia, which earlier had occurred as an extremely rare "phenodeviant" of our stock of Leghorn fowl, began to segregate in appreciable numbers in outcross families of this stock to three different breeds. When two of these families were interbred, early embryo mortality as well as incidence of cyclopic embryos rose sharply. The data as a whole seem to admit of only one interpretation, viz., that cyclopia homozygotes may survive as normal overlaps and that penetrance increases *pari passu* with the elimination of protective modifiers or their becoming heterozygous.

The foregoing digression is not aimed at the author of the book under discussion. For, he does not offer his well-reasoned propositions as a panacea for all the problems involved in "fitness." The intention was a

word of caution to the reader: the important suggestions put forward by Professor Lerner are in part born out by experimental evidence; in other respects they will stimulate new inquiries; but they should not be understood to suggest that all equilibrating events (whether in individuals or in populations) are based on heterozygosity. The versatility of nature confounds such ready generalizations. One must be grateful to Professor Lerner for the clear survey he has given us of one of the avenues by which selection accomplishes its ends. His discussion should go far in stimulating new attacks on this old and important problem.

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

Editorial Office, The American Naturalist
Box 2, Schermerhorn Hall, Columbia University
New York 27, N. Y.

Beale, G. H., 1954. The Genetics of Paramecium aurelia. 179 pp., 11 figs. $2.50. Cambridge Monographs in Experimental Biology 2, Cambridge University Press, Cambridge.

When Sonneborn discovered mating types in *Paramecium aurelia*, he predicted that the genetics of this form should now advance "rapidly into a systematic, coherent body of knowledge in close touch with the rest of genetic science" (PNAS 1937, p. 385). This prophecy has certainly been fulfilled in a striking way. Beale's monograph presents the factual basis for this fulfillment with the expected emphasis on the relative roles of nucleus and cytoplasm in paramecium heredity. This is the most complete account of modern paramecium genetics to appear since Sonneborn's review in 1947 (Advances in Genetics) and, of course, contains much new material including some not previously published. Two introductory chapters orient the general reader and acquaint him with the material and specialized methods of paramecium genetics. There follow four chapters, three on the major genetic characters (killer, antigen and mating type characters) and a final, concluding chapter. With the exception of a discussion of dauermodifications most work antedating the discovery of mating types is not treated. The author might have extended his coverage of this earlier material (e.g. the problem of aging is given one short paragraph).

The serious general reader will find this book informative and stimulating for it covers the subject matter well and is liberally, if not excessively, fertilized with opinion, hypothesis and discussion. The specialist in protozoan genetics will find the bibliography up-to-date, and the text well documented with tabular data but not encyclopedic in coverage.

CHARLES B. METZ

Burton, Maurice, 1954. Animal courtship. 267 p. ill. $4.00. Frederick A. Praeger, New York.

A valuable compendium of observations on courtship and mating patterns in various groups of the animal kingdom, written in a simple and engaging fashion by a Deputy Keeper of Zoology at the British Museum of Natural

History. The coverage is extensive, including both the older and newer literature. Regrettably, no bibliography is given. As to interpretation, the author views modern theories of evolution with some scepticism. His point of view is epitomized in the following statement: "There is in all living matter a creative drive, of which the reproductive drive is not necessarily the most compelling part, and courtship can be the expression of both these drives."

TH. DOBZHANSKY

Buzzati-Traverso, A. A. (Editor), 1954. Symposium on genetics of population structure. 141 p. $2.30. Published for the secretariat-general of the International Union of Biological Sciences by Libraria Internazionale Garzanti, Palazzo Universitario, Pavia, Italy.

This volume contains the texts of papers presented in the symposium held in Pavia, August 20-30, 1953, which was devoted in the words of the editor, "to those aspects of the genetics of quantitative traits that are of significance in population studies." The data papers, all printed in English are by G. Clayton, J. A. Morris and A. Robertson (University of Edinburgh); D. S. Falconer (Edinburgh); R. E. Scossiroli (Pavia); B. Wallace (Biological Laboratory, Cold Spring Harbor, New York); J. Clausen (Carnegie Institution, Stanford, California); H. Lewis (University of California, Los Angeles, California) with discussion and summaries by Th. Dobzhansky (Columbia University); I. M. Lerner (University of California, Berkeley, California); B. Wallace (Cold Spring Harbor); C. Epling (University of California, Los Angeles, California); A. Buzzati-Traverso (Pavia) and J. B. S. Haldane (London).

Clarke, L. George, 1954. Elements of ecology. 534 p., ill. $7.50. John Wiley & Sons, New York.

The book describes the relationships between organisms and their surroundings in a clear and concise way. The first chapter is devoted to describing the scope of the science of ecology together with some fundamental concepts such as the meaning of the word environment. In the next seven chapters the author considers particular aspects of the environment including temperature, light, water and nutrients. The remainder of the book is devoted to more complex topics such as the community, ecological succession and fluctuation, and finally the dynamics of the ecosystem. The author has succeeded in making a complex subject intelligible to the reader even if he has little or no previous knowledge of ecological principles. Moreover, there are a large number of diagrams and black and white photographs which help to make the more difficult points clear. The book is particularly suitable for naturalists and students beginning to take up ecology. There is also a good modern literature list at the end of the book which will be of help to advanced students as well as beginners.

P. M. SHEPPARD

Congdon, Russell T., 1955. Our beautiful western birds. 408 pp., ill. $9.00. Exposition Press, Inc., New York.

An illustrated, anecdotal account of birds watched and photographed in Washington, Oregon, California, and Churchill, Manitoba. There is little of scientific value, although a brief account of a raven who opened milk bottles and drank the cream recalls the well-publicized story of the spread of a similar habit among some British and Dutch passerines.

Lea, D. E., (2nd Edition), 1955. Actions of radiations on living cells. 416 p., ill. $6.00. Cambridge University Press, New York.

Lea's valuable book, which treats the elementary actions of ionizing radiations on living cells, including viruses, genes and chromosomes, is again available in its original form. The editor, Dr. L. H. Gray, has added to this edition the revisions and additions which Lea had made in his own copy of the 1946 edition before his death.

Macan, T. T., and E. B. Worthington, 1954. Life in lakes and rivers. 272 p., 45 color photographs, 68 black and white photographs, 20 maps and diagrams. $4.50. Frederick A. Praeger, Inc., New York.

Life in lakes and rivers is number 15 in the New Naturalist Series. The authors deal with such varied topics as the classification of lakes and rivers, apparatus used in fresh water biology, the effects of pollution, over-fishing and under-fishing, sewage purification and even the biology of organisms living in water pipes. The text is clear and simple and can be understood by readers with no biological training. Most of the book is concerned with British lakes and rivers. However, because the authors are more concerned with principles than examples, the book is of interest to naturalists everywhere and not only those acquainted with the Flora and Fauna of Britain. Moreover, there is much useful information for the fisherman, the fish cultivator, people interested in nature conservation and even the water engineer. The color photographs are good and add much to the pleasure that can be obtained from this excellent book.

P. M. SHEPPARD

Rand, Austin L., and Emmet R. Blake, 1954. Birds the world over. 96 p., ill. $1.50. Chicago Natural History Museum.

A guide to the Hall of Habitat Groups of Birds at the Chicago Natural History Museum. Each exhibit is illustrated by a photograph, some in color, and a numbered outline diagram for identifying the species shown. Informative notes on the birds are given. An introductory section is devoted to the zoogeographical regions upon which the floor plan is based. This booklet should be of general interest to students and amateur ornithologists.

Ritter, William Emerson, 1955. Charles Darwin and the golden rule. Compiled and edited by Edna Watson Bailey. 400 p. $5.00. Storm Publishers—Science Service, New York.

Drawn from manuscripts unpublished at the time of Dr. Ritter's death, skillfully and sympathetically selected and arranged, here is a volume to justify the publisher's hope that it may be "a useful step toward bridging the gap between science and the humanities." That this gap is dangerous and costly in terms of human values, even perhaps of human destiny, seems clear enough.

Whatever figure is used to describe it—gap, wall, or warfare—the situation must be resolved by genuine and profound mutual understanding. Not too much can be expected from casual dips across the barrier. The experimentalist who happens to enjoy good music needs more than that to prove that he is a humanist, and the same principle applies to the philosopher who has only a verbal knowledge of scientific experience. The synthesis, if it comes, must be generated by those who have been nurtured in both camps, who have two mother-tongues. Such a man, on the evidence of what he has written, William Ritter must have been. He was at home in each of two subcultures, knew their values and weaknesses, and worked to salvage and harmonize what was best in both.

With the simplicity of greatness, he leads right to the essence of the matter, which is the unity of the universe—"not by explicity rejecting (supernaturalism)..but by such..(a) conception of nature that there shall no longer be any need or room for supernaturalism." To this end he might have quoted the dream of Peter, the Rock, wherein the dreamer was adjured "What God hath cleansed, that call not thou common."

It will be gathered that the book is Ritter. But it is by happy circumstance built about an interpretation of Charles Darwin that does the great naturalist better justice than he often receives. Better justice it is, certainly than Darwin's ideas on morality had from his devoted bulldog, Huxley. For Darwin was deeply conscious of the binding power of the ethical sense in man. Ritter reminds us that Huxley, in his Romanes lecture, had much to say of the struggle for existence, but said nothing of natural selection which in Darwin's view must inevitably lead to the evolution among social, sensitive and intelligent beings of some such concept as the Golden Rule of Confucius and Jesus. Had the critics of "Social Darwinism" read more of the master and less of his disciples, they might at least have had the grace to coin a different synonym for ruthlessness.

After giving us, in the first third of his book, a remarkably fair appraisal of Darwin's views, the author trains his scientific resources and humanistic insight onto the evolution in humanity of its ethics, economics, reproduction, intelligence, science and religion. It is especially interesting, after reading two recent and excellent books, Weston LaBarre's THE HUMAN ANIMAL and Carleton Coon's THE STORY OF MAN, both of which deal in the modern mode with sex, robustly and without inhibition, to see how effectively William Ritter gets the job done within the bounds of a delicacy and restraint now often considered passé.

PAUL B. SEARS

Runes, Dagobert D. (Editor), 1955. Treasury of philosophy. 1280 p. $15.00. The Philosophical Library, New York.

The energetic editor and publisher have assembled brief excerpts, with biographical comments, from nearly 400 writers born between 625 B.C. (Thales) and 1905 (Sartre) including besides the usual Greeks and Europeans, Chinese, Hebrew, Arabic, Indian, and American scholars. The editor's definition of philosophy is "search for the indefinable." As a consequence, the scientists included (and there are many) are in general represented by statements about meanings and feelings rather than about knowledge. Thus from Charles Darwin we have the famous autobiographical passage about his religious views, and from Percy Bridgman thoughts about the social responsibility of the scientist. The table of contents reminds one of those primitive paintings of the creation where lion and lamb consort peacefully. There we have Allen (Ethan) and Anaxagoras; Engels and Empedocles, Lenin and Lessing, Maritain and Marx (Karl), and many other alphabetic anomalies. It's a bargain.

L. C. DUNN

Wigglesworth, V. B., 1954. Cambridge monographs in experimental biology I: The physiology of insect metamorphosis. 152 p., ill. $2.50. Cambridge University Press.

Professor Wigglesworth is one of the pioneers in the field of insect endocrinology and his extensive research on the metamorphosis of Rhodnius has been reviewed in this publication. In addition he has briefly summarized and commented on the literature dealing with the endocrine functions of the neurosecretory centers of the brain, the corpus allatum, the thoracic gland of various insects, and the ring gland of the Diptera. The author has considered insect metamorphosis "a special case of a general phenomenon, which is polymorphism in the widest sense." Polymorphism may be due to differences in the genotypes of individuals or different environments. It is in the latter sense that insect metamorphosis is comparable to polymorphism since genotypically similar cells and tissues of a developing insect are subjected to changing internal environments with the result that different structures are formed as well as larval, pupal and adult stages. Among other examples presented in support of this concept an interesting comparison has been made between the differentiation of tissues of an individual and caste formation in termites through substances called "social hormones." This monograph will prove useful for students of insect physiology as well as those interested in problems of growth and differentiation.

M. T. M. RIZKI

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